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The vascular pattern of the rat cochlea was demonstrated by the aid of Berlin Blue (Prussian Blue) stain. The vascular anatomy is similar to other mammals. The infrequent occurrence of the ensel of the spiral lamina under the organ of Corti is interesting in respect to its presumed importance for the oxygen supply to the hair cells. In the place where the vessel of the basilar membrane usually lies when present, large unmyelinated axons are often seen throughout the cochlea. In the rat, all radiating arterioles supply and collecting veins drain all capillary areas. The vascular pattern of the cochlea is well demonstrated at the basal end but more sparse in apical parts of the cochlea as in other mammals. The rat cochlea is somewhat more difficult to dissect than the cochlea in other mammals due to its small size, but the dissection of different parts of the cochlea was achieved without major problems.

The anatomy of the cochlear vasculature of the rat has been described in the early literature (Asai 1908, Nabeya, 1923). Recently, Wicke (1978) has studied the spiral ganglion blood supply in the rat cochlea. Hodde et al (1977) and Mioudonski et al (1978) have also presented excellent treatises on the rat vasculature. While the overall vascular pattern was quite well demonstrated by their micro-corrosion cast technique, detailed information on particular vessels of the spiral lamina was not given. The aim of the present investigation was to complete the picture of the cochlear vascular pattern in the rat.

The rat is a very commonly used animal in research. Both normotensive and hypertensive rats have been used as models in cardiovascular studies (Hallböök & Folkow 1974, Ingvar 1974, Borg, 1976). Devine & Simpson (1967) used the rat vascular system to study the structure of sympathetic neuromuscular contacts. The autonomic nerve supply to the inner ear vasculature of the rat has also been investigated (Spoendlin & Lichtensteiger 1966). Further, small blood vessels of various parts and tissues in the rat have been a subject of research by means of electronmicroscopy (Rhodin 1977). The fact that the rat is resistant to middle ear infection has an accessible inner ear and can be trained by behavioural techniques has made it a very popular subject for electrophysiological (Møller 1970) as well as psycho-physical measurements.

## MATERIAL AND METHODS

Eight rats, each weighing approximately 250 g, were used in these experiments. The demonstration of the cochlear blood vessels was achieved by a previously described method (Axelsson, 1968, 1971, 1972, 1973, Axelsson & Lind 1973) which in summary contains the following preparative steps: Anesthesia with intraperitoneal administration of urethane, perfusion of the vascular system with Ringer's solution under hydrostatic pressure, injection of a contrast, Berlin Blue (Prussian Blue) solution under the same hydrostatic pressure, fixation of the cochlea, decalcification, washing, dehydration, storage in glycerine, microdissection of the cochlea using the stereo-microscope, photographic registration of the microscopic findings using the light and phase contrast-microscope (Leitz Orthoplan).



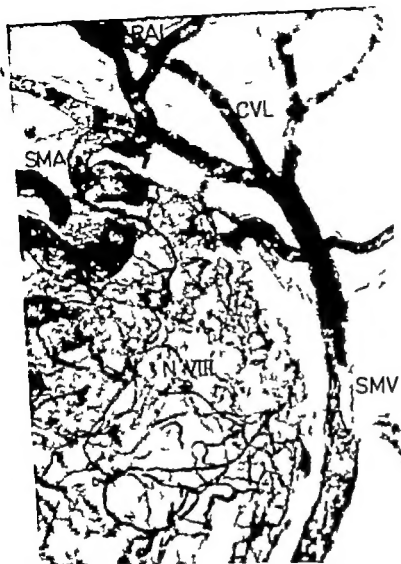


Fig. 2 Rat cochlea, transverse section. Modiolus, turn I SMA, spiral modiolar artery branching into two RAL, radiating arterioles. Branches of CVL, collecting venules, emptying into SMV, spiral modiolar vein, N VIII acoustic nerve.

pig but run a more serpentine winding course. The rat cochlea is drained by the spiral modiolar vein (SMV) which also runs a spiral course around the modiolus from the apex to the base (Fig. 1). SMV is situated in the basal central angle of the scala tympani below the spiral ganglion. SMV receives many collecting venules (CVL) from the external wall the spiral lamina and limbus vessels and the capillary areas in the modiolus. In one animal a vessel was found running from the modiolus through the scala tympani to the external wall.

This vessel is previously termed the "suspension vein" when found in the guinea pig. Similar to the guinea pig the "suspension vein" could be demonstrated in the "hook" region near the round window.

#### *Spiral lamina*

The following vessels can be identified in the spiral lamina. Radiating arterioles RAL, collecting venules CVL, vessel of the tympanic lip VSTL, limbus vessels LVS vessel of the basilar membrane VSBM.

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Fig. 4 Rat cochlea, spiral lamina, turn 1.5 VSTL, vessel of the tympanic lip which often forms two spiral vascular borders at the tympanic lip. A loop formation of vascular connections between these two border vessels is often seen, OHC = outer hair cells.



Fig. 5 Rat cochlea, spiral lamina, turn 1 VSTL, vessel of the tympanic lip. VSBM = vessel of the basilar membrane. Channel without contrast (arrow).

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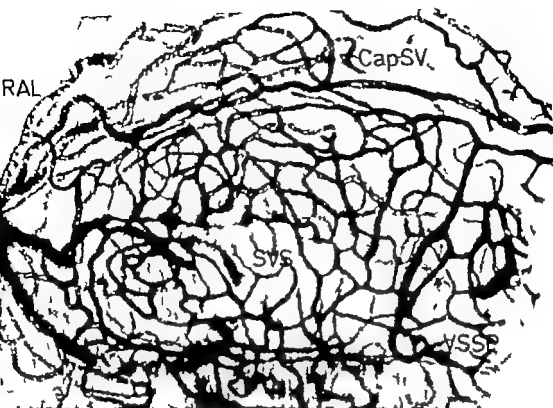


Fig. 9 Rat cochlea, extreme basal end RAL, radiating arterioles, CapSV capillaries of the scala vestibuli

SVS stria vascularis, VSSP vessel of the spiral prominence

dially running CVL and these in turn often run in a fairly short distance radially before they empty in a large spirally running collecting venule. Some of the CVL ST draining the stria vascularis and the arterio-venous anastomoses run directly to such a large collecting venule. In this way the appearance of the scala tympani is much less vascularized particularly in its basal portions, than in other mammals.

#### Apex

Apical parts of the rat cochlea are characterized by the same simplification of the vascular pattern that was found in other mammals (Fig. 8). Despite the general simplification most of the regularly occurring vessels can be demonstrated here.

#### Basal end

The vascular pattern of the basal end is very similar to that in other mammals investigated. The basal end is supplied by the vestibulo-cochlear artery and drained by the vein of the round window (Fig. 1). In the region between the round window and the oval window the "hook" region the radiating arterioles coming from the vestibulo-cochlear artery enter the stria vascularis with a more oblique course. Other branches from the vestibulo-cochlear artery run in a retrograde apical direction after turning around the extreme basal end of the cochlea. These retrograde obliquely-running radiating arterioles also supply the capillary areas in the external wall. In the region where the radiating arterioles from both directions merge between the windows an anastomosing



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5 min following HRP injection were required before Karnovsky (1967) found capillary basal lamina densely stained. Although transport of HRP in muscle capillaries was primarily by means of pinocytosis, intercellular cleft transport also occurred.

In contrast with other permeability studies, Hako & Hilding (1971) have reported that HRP is transported across strial capillaries by a route between endothelial cells and that macropinocytotic vesicles are absent within strial endothelia of both Ames-Waltzer mice and normal Swiss strain mice.

Yamamoto & Nakai (1964) studied strial capillary transport in the Guinea pig using iron dextran. At no time point after injection (10 min to 77 h) was the tracer observed in capillary basal lamina, although membrane bound accumulations were frequently found within endothelia. While these authors determined the size of iron dextran to be 100 Å in diameter, others have shown that the diameter of this tracer ranges from 20–70 Å (Muir & Goldberg, 1961; Richter, 1959). Molecules this size should be transported across strial endothelia if indeed the HRP results are sound. In fact, if one considers that the transport rate of HRP (mol. diam. 55 Å) is more rapid through strial than muscle capillaries and that transport in both cases primarily involves pinocytosis, then it is difficult to account for the iron dextran results in light of the successful transport of molecules as large as ferritin (mol. diam. 110 Å) across muscle endothelia (Bruns & Palade, 1968). Clearly the literature on strial capillary permeability is in conflict.

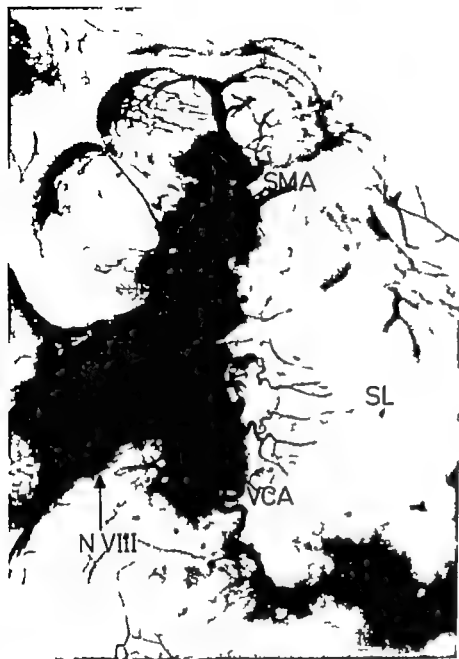
It should be noted that the use of HRP as a tracer to study strial capillary permeability has certain drawbacks. For example, the enzyme HRP was noted by Duvall et al. (1971) to cause strial capillary alterations consisting of intraluminal protrusions of endothelial cell membranes and pores within capillary walls. Cell protrusions have been known to occur in injured cells (Trump & Arstila, 1974). The artifactual production of pores in strial capillaries could have resulted in the release of HRP

to the basal lamina. Such changes do not occur in adjacent spiral ligament vessels where no HRP exists. Whether strial capillary alteration is a manifestation of HRP toxicity to select portions of the inner ear is not clear; however, it has been found that perilymphatic perfusion of HRP *in vivo* is acutely ototoxic (Ross et al., 1977).

Other problems of HRP use include: (1) the enzyme must retain its catalytic activity until and throughout incubation; (2) quantitative analysis of transported tracer substance cannot be performed since a) the HRP itself is not directly visualized and b) the quantity and quality of the reaction product is dependent upon the quality of both the enzyme preparation and incubation medium, the penetration of the medium into the tissue and the duration of the incubation; (3) histamine, which affects vessel permeability and strial capillary blood flow, is released in some species in response to HRP injection; (4) an endogenous peroxidase reaction exists which may interfere with the interpretation of experimental findings.

These problems are especially troublesome when evaluating capillary permeability changes in experimentally induced pathologies. For example, Duvall et al. (1974) used the tracer substance HRP to evaluate strial capillary permeability after intense noise exposure. They suggested that some of their unusual experimental results may have been due to the degradation of the protein HRP by lysosomal enzymes. Of course, if HRP is inactivated by lysosomal hydrolases, then the electron dense HRP reaction product, which indicates the presence of HRP, will not be produced. Therefore, the transport of the tracer cannot be determined.

Certainly then, HRP and similar enzymatic tracers pose serious problems when used as a means to gauge strial capillary transport during pathologic as well as normal conditions. A reliable method of evaluating the function of strial capillaries without the limitations imposed by the use of an enzymatic tracer would be beneficial.



*Fig 1* Rat cochlea, apico-basal paramodiolar section. SMA spiral modiolar artery VCA vestibulo-cochlear artery SL spiral lamina, N VIII acoustic nerve

## RESULTS

A more or less good contrast injection of the cochlear vascular system was achieved in all animals. As in other mammals some parts of the capillary areas are often less well injected than others in the immediate vicinity. This appears to be more the result of technical defects than due to pathological conditions in the vascular system.

The rat cochlea has three turns. In principle the blood supply to the rat cochlea appears to be very similar to that of the guinea pig

The arterial supply is maintained by a large artery the spiral modiolar artery (SMA) running spirally around the modiolus from the base to the apex (Figs 1 and 2). SMA is situated between the acoustic nerve and the spiral ganglion at the level of the scala vestibuli. Radiating arterioles (RAL) leave the spiral modiolar artery to supply the capillary areas in the modiolus in the scala vestibuli and the scala media and in the spiral lamina and limbus (Fig. 3). RAL do not form spring coils as previously described in the guinea

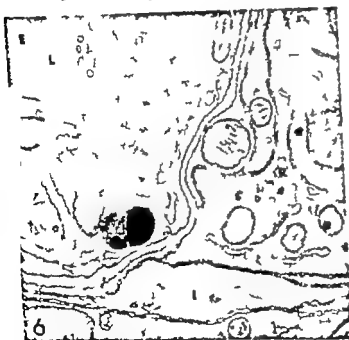
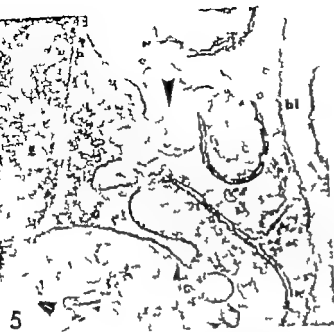


Fig. 5 Large arrow indicates open-mouthed vacuole containing large amount of ferritin on basolateral front. Median arrow indicates apical tubular invagination containing ferritin. Double arrow indicates fusion of two labeled pinocytotic vesicles. Ferritin molecule can be seen in basal lamella (small arrow) is post injection. Glutaraldehyde and osmium fixed. Unstained. Mid portion of cochlear duct.  $\times 90,025$ .

Fig. 6 Control axonal (unmyelinated) showing light cell (R) with abundant supply of endogenous ferritin-like granules. Osmium fixed. Unstained.  $\times 39,600$ .

axicles were observed within endothelial cells (Figs. 5, 7, 8 and 9). Labeling of any pinocytotic vesicles was limited to no more than a few molecules of ferritin. In any section the number of such labeled vesicles per endo-

thelial cell varied from none to a few, with no discernible relation to time after injection or area of cochlear duct. At times the number of unlabeled vesicles was great. Although endothelial vesicles opening to the tissue front were

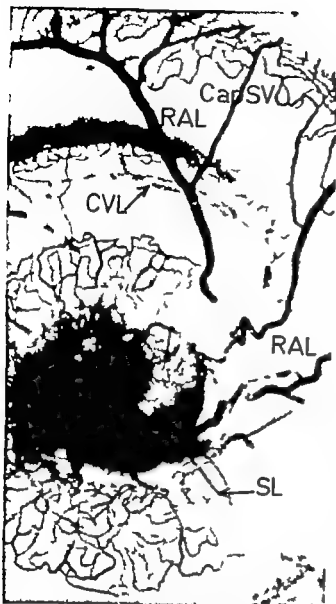


Fig 3 Rat cochlea, transverse section turn apical view RAL radiating arterioles supplying the external wall CapS capillaries of the scala vestibuli CVL collecting venules draining the external wall SL, spiral lamina.

*Radiating arterioles and collecting venules (RAL-CVL)* RAL supplying the capillary vessels are branches from the spiral modiolar artery. They run centrifugally over the spiral lamina (Fig 3). Each RAL supplies a rather small segment. When RAL reach the vessel of the tympanic lip or the vessel of the basilar membrane they turn off squarely in T junctions or build up arcades. CVL drain the vessel of the basilar membrane, the vessel of the tympanic lip and the limbus vessels and run

centripetally over the spiral lamina to empty in the spiral modiolar vein.

*The vessel of the tympanic lip (VSTL)* VSTL is a well-supplied and well-drained vessel which is found throughout the cochlea and is typically well injected by contrast. It forms one or often two spiral marginal vascular borders in the tympanic lip (Fig 4). There is often a system of vascular loops connecting these two inner spiral vessels in addition to the more central loops in the spiral lamina. In this way the vessels appear to form a capillary net in the spiral lamina which end peripherally in one or two vascular margins of VSTL.

*The limbus vessels (LVS)* LVS make up a capillary network peripherally in the spiral limbus. The loops are sparse throughout the entire cochlea. Different branches from the same radiating arterioles and collecting venules are provided for the VSTL and VSBM and the LVS and there are no direct connections between those spiral vessels and the LVS. The blood supply to the spiral limbus appears poor in the rat in comparison with other mammals.

*The vessel of the basilar membrane (VSBM)* VSBM is not very often seen. However large uninjected channels resembling the previously termed "avascular channels" are almost always seen, especially in the basal turn under the tunnel of Corti where VSBM usually lies when present (Fig 5). In cases where the vessel was present it was a single injected vessel without branches. The non-contrast injected channels themselves appear to be long—extending over a whole turn—with very few supplying radiating arterioles or draining collecting venules. At the so-called T junction where supplying and draining radiating arterioles and collecting venules join VSBM one can sometimes see an injected VSBM within a perivascular space running spirally in one direction while a channel without contrast continues spirally in the other direction (Fig 5). Nothing was ever seen at the T junction to inhibit the injection of Berlin Blue.

The vestibular and tectorial membranes are



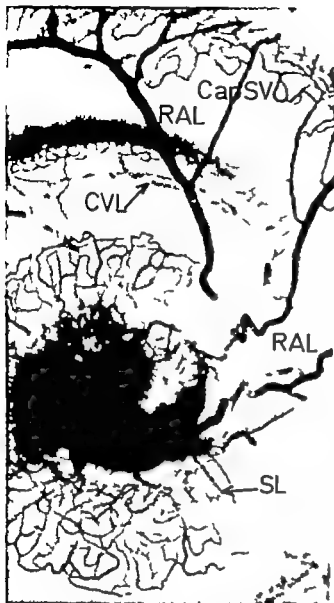


Fig. 3 Rat cochlea, transverse section turn 2 apical view. RAL, radiating arterioles supplying the external wall. CapSV, capillaries of the scala vestibuli. CVL, collecting venules draining the external wall. SL, spiral lamina.

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The vestibular and tectorial membranes are

small fraction of the whole vesicle population. Within the basal lamina only isolated or small groups of 2-3 ferritin molecules were scattered along the circumference of the capillaries. These findings are surprisingly similar to those of the present study. However, despite the similarities in capillary structure and ferritin transport, the transendothelial passage of HRP is vastly different between stria and cortical thymic capillaries. Raviola & Karnovsky (1977) demonstrated that the amount of HRP transport across cortical thymic capillaries is comparable to that of ferritin. That is, from 2-20 min post injection, only limited vesicular transport of the enzymatic tracer occurred through endothelia; vascular adventitia was free of peroxidase reaction product. Apparently then, the transport of both ferritin and HRP is similar for a given capillary bed provided that the micropinocytotic transport system is the sole means of transendothelial passage. The similar transport characteristics of HRP and ferritin agree well with the conclusions of Renkin (1964).

Another blood-tissue barrier has been reported which is similar to the thymic one (Gershon & Burzstajn 1978). The myenteric plexus is supplied with continuous-type capillaries. Using HRP, Gershon & Burzstajn (1978) demonstrated minimal transport of the tracer, presumably by the pinocytotic pathway, since intercellular cleft transport was impossible due to tight junctional seals. Extravascular accumulation of the HRP reaction product in the myenteric plexus was undetectable. Raviola and Karnovsky reported for cortical thymic tissue. However, in both tissues—thymus and myenteric plexus—it was concluded that some transport has occurred because perivascular phagocytes contained vacuolated tracer substance.

In the present study, the ferritin results appear to indicate the existence of a blood-stria barrier which is closer in nature to the blood-thymic and blood-myenteric plexus barriers than to the blood-brain barrier.

Another phenomenon which might account

for the observed minimal transport of ferritin across stria capillaries is the phagocytotic activity of stria endothelial cells. Within these cells were observed large vacuoles and multivesicular bodies containing ferritin similar to those reported by Raviola & Karnovsky (1977). Apparently one means of such vacuole formation was by pinocytotic vesicular fusion, since with increasing time, larger and denser ferritin-containing bodies (presumably lysosomes) were present. It appeared that once ferritin conglomerates formed, the ferritin was immobilized within endothelia until degraded. In essence then, the phagocytotic activities of stria endothelia in conjunction with minimal pinocytotic transport and endothelial tight junctions contribute to the formation of a blood-stria barrier at least for molecules the size of ferritin.

Previous studies have suggested that intermediate cells may serve as macrophages within the stria. Such cells have been found to have open-mouthed pinocytotic vesicles and micropinocytotic vacuoles labeled with HRP reaction product after a sufficient interval of time following HRP administration (Duvall et al. 1971; Winther 1971). Also at the light microscopic level, Nomura (1961) found that intravenously injected trypan blue was phagocytized by certain stria cells scattered about the capillaries. Similarly transported tracer substances within cortical thymic and myenteric plexus blood-tissue barrier systems were incorporated within perivascular phagocytic cells (Raviola & Karnovsky 1972; Gershon & Burzstajn 1978). The possibility exists that in this study, some ferritin-like particles within the tight cells of experimental animals may be exogenous ferritin that is perhaps the cytoplasm of these cells serves as a sink for transported exogenous ferritin. However, the fact that such particles were located for the most part, free within the tight cell cytoplasmic matrix, argues against an exogenous source, since macromolecules of this size are not able to enter free cytoplasm. Further, it should be noted that there were no obvious differences



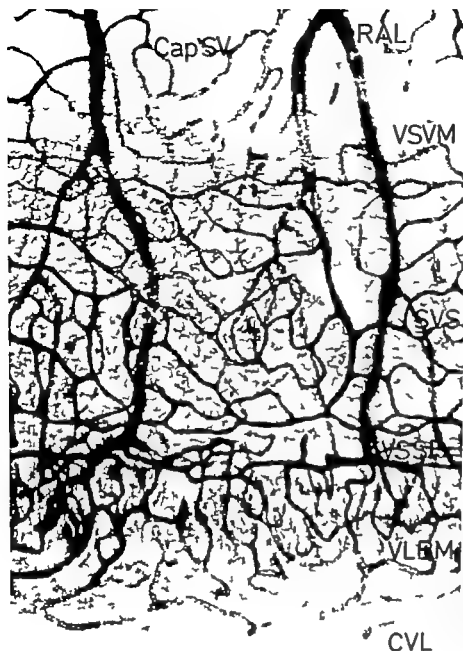


Fig 6 Rat cochlea external wall turn ... RAL, radiating arterioles supplying SVS and VSSP. CapSV, capillaries of the scala vestibuli. VSVM, vessel at the vestibular membrane. SVS, stria vascularis. VSSP, vessel of the spiral prominence. VLM, venules at the basilar membrane. CVL, collecting venules.

avascular which is in agreement with the principal vascular pattern of the other mammals previously examined

#### External wall

The following vascular structures can be demonstrated in the external wall

*Scala vestibuli radiating arterioles* RAL, capillary net of the scala vestibuli CapSV, vessel at the vestibular membrane VSVM

*Radiating arterioles (RAL)* RAL originate from the spiral modiolar artery and supply all

the capillary areas in the external wall (Fig 3). In general RAL are large vessels which run a serpentine centrifugal course before they straighten out and ramify. RAL seem to maintain the same calibre from base to apex. The most frequently observed pattern of arterial supply of the stria vascularis is for the RAL to run external to the stria bed and then connect directly to the basal marginal stria vessel. The basal marginal vessel usually was more prominent than other capillaries in the stria vascularis. More RAL branches seem to supply

## ZUSAMMENFASSUNG

Enzymatische Tracermethoden für die Erforschung poröser und pathologischer Kapillärtransports der Stria stellen verschiedene Probleme vor. Die Verwendung optischer Elektronentracer kann viele von diesen Problemen vermeiden. Eisen-dextran (mol. Gew. 20-70 Å) und Ferritin (mol. Gew. 110 Å) wurden intraveneös eingespritzt, und die Mäuse wurden nach Zwischenzeiten von 1, 2, 5 und 24 Stunden geopfert. Die Eisen-dextran-Ergebnisse waren ungewöhnlich, weil der Tracer von 1 bis 5 Stunden nach der Einspritzung in der zytoplasmatischen Matrix der Endothelien zugegen war, aber gegen die 24. Stunde war er ausgeschieden worden. Kein transendothelialer Austausch wurde bemerkt. Die Ferritin-Ergebnisse widersprachen vorübergehenden Ergebnissen, die Meerrettich-peroxidase benutzten. Der Transport von Ferritin war unabhängig der verstrichenen Zeit maximal. Nur ein paar Moleküle wurden um die Basillarkapillarien herum gesehen. Die über Kapillaren übergeführten Moleküle waren dem anschließend mittels des mikrophotografischen Systems übergeben. Die Ergebnisse deuten eine Blut-Stria-Sperre an, die den Blut-Thymus- und Blut-Meningeal-Sperren ähnlich ist. Versuchs- sowie auch Kontrollversuche wiesen Lichtzellen der Stria auf die Perilymphatische Partikel in ihren zytoplasmatischen Matrizen auf. Diese Lichtzellen sind wahrscheinlich Zellen des retikuloendothelialen Typs. Ferritin könnte nutzbar sein, um die auf Gehörpathologien bezogenen Änderungen des Kapillärtransports der Stria abzuschätzen.

## ACKNOWLEDGEMENTS

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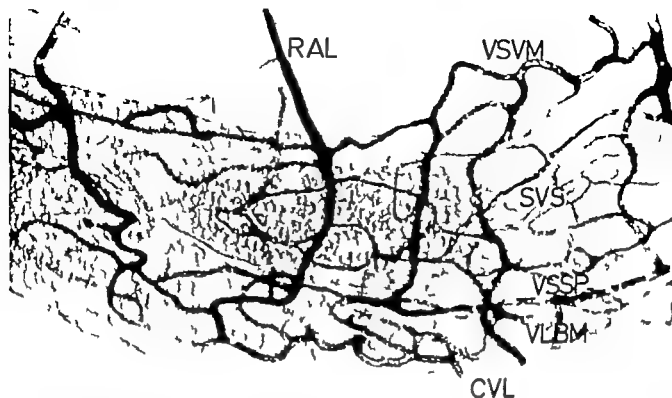


Fig 8 Rat cochlea turn 3 Apical simplification RAL radiating arterioles VSVM vessel at the vestibular membrane SVS stria vascularis VSSP vessel of the spiral

prominence VLBM venules at the basilar membrane CVL collecting venules

veloped in terms of number of loops in the middle turns. There is a simplification of the SVS in the third turn. In the basal turn the width of the stria is decreased and the loops are more tightly packed.

*The vessel of the spiral prominence (VSSP)* Parallel and basal to the stria vascularis runs VSSP (Figs 6 and 7). It is supplied by separate branches of the radiating arterioles of the scala vestibuli. VSSP is made up of spirally running segments which are irregular, i.e. long and short segments are interspersed. Very often VSSP ramifies into a double vessel. VSSP is often drained by a short branch which first travels above the spirally running segment and then loops downward toward the scala tympani into the collecting venule. For short distances there can be an absence of the VSSP. VSSP is approximately of the same calibre as the basal marginal stria vessel. There seems to be no difference with respect to vessel calibre apically and basally.

*Arterio-venous anastomoses (AVAS)* External to the stria vascularis and the vessel of the spiral prominence there are connections between the radiating arterioles in the scala vestibuli and the collecting venules in the scala tympani. These vessels consequently offer a by-pass possibility where the arterial blood in the scala vestibuli can be shunted over directly to the venous blood in the scala tympani.

*Scala tympani* Venules at the basilar membrane VLBM collecting venules scala tympani CVL ST

*Collecting venules-scala tympani (CVL ST) and the venules at the basilar membrane (VLBM)* CVL ST in the rat have a different appearance from many other mammals. Most of the venules make up a vascular net in the region of the attachment of the basilar membrane. Basally this vascular net has well defined spirally running segments of venules (VLBM) (Figs 6 and 7). This spiral VLBM border is drained by comparatively few ru-

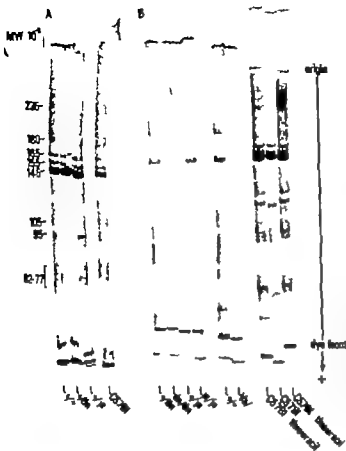


Fig. 2. Gels grouped together were subjected to electrophoresis at the same time. Remains of the stacking gels can be seen at the top of the picture. 7% gels stained with Coomassie blue. (A) Normal tectorial membranes from a variety of genetic backgrounds. The molecular weights of the major bands are labelled, and were estimated using the relative mobilities of standard proteins run at the same time as the membrane samples. (B) Tectorial membrane samples from mutants and their normal littermates, and from thioacetamide-treated and control C57Bl mice. No significant differences in the banding patterns can be detected.

method used was based on that described by Laemmli (1970) and Maizel (1971).

A stock solution was added to each sample to bring the final concentration to 2% SDS, 0.1 M Tris-HCl (pH 7.0) and 5%  $\beta$ -mercaptoethanol, and the density was increased by adding about 10% sucrose. This was heated to 100°C for two minutes to ensure denaturation of the proteins. The numbers of samples examined were as follows: C57Bl-18, C57Bl+thiouracil-13, je/je-6, +/je-7, dn/dn-6, +/dn-7, Va/+6, +/+7.

Gels were cast in glass tubes 9 cm long, with an internal diameter of 0.27 cm, and were prepared by standard procedures as described by Davis (1964) and Ornstein (1964). Seven per cent acrylamide running gels and 3% stacking gels were used, both containing 0.1% SDS and

with an acrylamide to bisacrylamide ratio of 30:0.8.

The discontinuous buffer system of Laemmli (1970) was used with Tris-HCl in the running gel and Tris-glycine at the electrodes. The electrode buffer consisted of 25 mM Tris, 192 mM glycine and 0.1% SDS, and its pH was 8.5. Electrophoresis was carried out with a constant voltage and an initial current of 1.5 mA per gel, and was stopped after about two hours when the tracking dye reached a set point. The gels were then removed from their tubes and fixed and stained with Coomassie blue as described by Fairbanks et al. (1971).

Standard proteins of known sub-unit molecular weight were used to calibrate the gels, and the molecular weights of the proteins in the membrane samples were obtained by com-

net is formed. The stria vascularis is unusually well developed all the way to the extreme basal end in the rat cochlea and better developed than in the other mammals studied so far (Fig. 9). The capillary areas at the basal end are drained by collecting venules which empty into the vein of the round window.

## DISCUSSION

The reason for studying the vascular anatomy of the rat cochlea is the frequent use of this animal in experiments concerning hearing research. There has been a lack of systematic investigation of the complete cochlear vasculature in this animal. The contrast injection technique using Berlin Blue has proved to be a satisfactory method for studying vascular anatomy. It would be misleading, however, to suggest that the technique proceeds without difficulty. 2.5% Berlin Blue in distilled water alone gave a poor and uneven injection. A better, more even injection was achieved by dissolving the 2.5% Berlin Blue contrast powder in an 8% citric acid solution. Insertion of the plastic tube with perfusion and contrast was more easily performed in animals of 250 g than in smaller animals. Some vessels stained better than others which suggests that these routes offer less resistance to circulation than the less well injected vessels do.

The vascular supply in the mammals thus far studied by us—man, rhesus monkey, rabbit, chinchilla, guinea pig and rat—seems to be strikingly similar. Each of these animals, however, has particular anatomical details usually making it fairly easy to differentiate its cochlear vasculature from other mammals. In the spiral lamina, the vessel of the tympanic lip in most mammals can be described as a single vessel bordering the tympanic lip being supplied and drained in an arcadic fashion. In the rat, the vessel of the tympanic lip also forms a spiral vascular border in the tympanic lip but a parallel vessel or vascular loop forming a capillary net may also be found running more centrally. In this respect, the rat vessels

are similar to the open and curved vascular arcades found in the rabbit but different from other mammals.

The vessel of the basilar membrane was only occasionally injected by contrast. Most of the time it appeared as a channel without red blood cells and without injected contrast. So far, the vessel of the basilar membrane has only regularly been identified in the guinea pig and in man. As in the rat, it can be demonstrated by contrast in short sections in the chinchilla but not at all in the rabbit. Many authors have discussed the possible importance of the vessel of the basilar membrane for the oxygen supply to the organ of Corti. It is well known that this vessel is large during embryonic life, much larger than in adult life. It appears then that the vessel of the basilar membrane may be important for the development of the organ of Corti but less so for the maintenance of its oxygen supply during adult life, since it is often missing. The significance of the empty channels cannot be evaluated with the present technique. Probably it is a remnant of a blood vessel. It might be argued that in case of a particular need for blood supply to the organ of Corti, these channels could regain their red blood cell circulation and function as blood vessels.

The external wall is the region which appears to be most similar in different mammals with easily identified supplying and draining vessels to well-defined capillary areas. The most obvious difference between the rat and other mammals is found in the collecting venules in the scala tympani. In the rat, external wall there is a rich net of collecting venules in the region of the attachment of the basilar membrane but as the venules continue basally they often form a sparse, radially running network which is drained by spiral segments of larger collecting venules.

In one animal, a "suspension vein" was found running from the modiolus through the perilymph to the collecting venules of the scala tympani in the external wall. The suspension vein appeared to be of larger calibre

## THE ELASTIC PROPERTIES OF THE TYMPANIC MEMBRANE SYSTEM IN DIVERS AND NON DIVERS

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(Received April 19 1979)

**Abstract** The volume/pressure relationship of the tympanic membrane system was studied in 24 scuba divers. The results were compared with the results obtained from 98 non-diving subjects. Among persons with a good function of the Eustachian tube divers had an increased mobility of the tympanic membrane system as compared to non diving subjects. Divers with reduced function of the Eustachian tube did not differ from the non-diving subjects with reduced tubal function. Factors that might influence the volume/pressure relationship of the tympanic membrane system are discussed.

The past two decades have seen a great increase in the number of people engaged in commercial as well as recreational diving. In 1965 it was estimated that five million people in the United States were utilizing scuba diving equipment. This growing interest in scuba diving has increased the number of preferably young people diving with limited training and often too little knowledge of the risks involved. Thus, it seems to be of great importance to expand our knowledge of the physiology as well as pathophysiology of the ear in diving.

Otologic problems related to exposure to compressed gas environments are most often an effect of inadequate equalization between the middle ear and the ambient pressure. Diving implies rapid large and frequent changes in ambient pressure and it can be assumed that inability to equilibrate middle ear pressure with ambient pressure might affect the elastic elements of the tympanic membrane system i.e. the tympanic membrane,

ossicles ligaments and middle ear muscles. The elastic properties of the tympanic membrane system can be determined by its volume/pressure relationship.

Studies on normal subjects without diving experience have revealed a relation between the elastic properties of the tympanic membrane system and the function of the Eustachian tube. Subjects with a reduced tubal function had a more mobile tympanic membrane (Elner et al. 1971c). This was considered to be caused by an inability to keep the middle ear pressure equal to ambient pressure. With these results in mind we found it of interest to study to what extent repeated barotrauma might cause changes in the elastic properties of the tympanic membrane system in a group of experienced scuba divers.

The aim of the present study was (1) to compare the elastic properties of the tympanic membrane system in divers and non-divers and (2) to find out if there is a relation between the elastic properties of the tympanic membrane system and the Eustachian tubal function in divers also.

### METHOD

Quantitative studies of the pressure regulating capacity of the Eustachian tube were made

## AN EVALUATION OF NORMAL STRIAL CAPILLARY TRANSPORT USING THE ELECTRON-OPAQUE TRACERS FERRITIN AND IRON DEXTRAN

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(Received March 14 1979)

**Abstract** Enzymatic tracer techniques to study normal and pathologic stria capillary transport pose various problems. The use of electron opaque tracers can circumvent many of these problems. Iron dextran (mol. diam 70-70 Å) and ferritin (mol. diam 110 Å) were injected intravenously and the mice sacrificed at intervals of 1, 2, 5 and 24 h. The iron dextran results were unusual in that from 1 to 5 h after administration the tracer was present within the cytoplasmic matrix of endothelia, but by 4 h it had been cleared out. No transendothelial exchange was noted. The ferritin results were in conflict with previous results using horseradish peroxidase. Transport of ferritin was minimal regardless of time sacrificed. No more than a few molecules were scattered about the capillary basal lamina. Those molecules transported across capillaries were apparently delivered by means of the micropinocytotic system. The results suggest a blood-stria barrier similar to the blood-thymic and blood-myenteric barriers. Experimental as well as control animals exhibited stria light cells which contained ferritin-like particles within their cytoplasmic matrices. These light cells are probably reticulo-endothelial type cells. Ferritin may be useful to gauge stria capillary transport alterations associated with auditory pathologies.

The functional role of the stria vascularis which includes the production of the endocochlear DC potential and ionic composition of the endolymph is important for normal inner ear function (Honrubia & Ward 1969; Konishi et al. 1966). The stria vascularis lines the lateral wall of the cochlear duct and is composed of three cell layers termed marginal, intermediate and basal (Smith 1957). The tissue is effectively a separate compartment sealed off from the endolymphatic space and spiral ligament by zonulae occludens which occur between the cell membranes of abutting marginal cells and abutting basal cells

(Jahnke 1976). This tight junctional seal around the stria limits the nutrient inflow from neighboring sources; therefore the high stria metabolic requirements (Chou & Rodgers 1962) must be met essentially by intrinsic capillary networks (Reale et al. 1975). Thus the permeability functions of the stria capillaries are paramount when considering the survival and function of the stria cells; accordingly stria capillary permeability is important for normal cochlear function.

Tracer molecules have been used to map the transport of materials through stria capillaries (Yamamoto & Nakai 1964; Duvall et al. 1971; Winther 1971; Gorgas & Jahnke 1974). Tight junctions have been found to exist between stria endothelial cells as have been found to exist between capillary endothelia of most CNS tissue (Duvall et al. 1971; Gorgas & Jahnke 1974; Reese & Karnovsky 1967). Tight junctional seals are known to prevent the passage of lipid insoluble materials through intercellular clefts. Therefore passage of lipid insoluble materials through the capillaries of the stria vascularis appears to be limited to the micropinocytotic vesicular system (Duvall et al. 1971; Gorgas & Jahnke 1974). By means of the vesicular system transport of the tracer molecule horseradish peroxidase (HRP; mol. wt. 43 000; mol. diam. 55 Å) is very rapid. As early as 1-2 min following HRP injection Duvall et al. (1971) observed the HRP reaction product distributed through stria capillary basal lamina. In muscle however more than

## VIBRATION MEASUREMENT OF THE HUMAN TYMPANIC MEMBRANE—IN VIVO

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(Received March 12, 1979)

**Abstract** The vibration patterns of the human eardrum in vivo have been recorded by time average electronic speckle pattern interferometry—ESPI. The necessary stability was achieved by shortening the exposure time of each TV frame. The amplitude- and phase-distribution as mapped across the drum by phase-modulation techniques which also could show the vibrations as slow motion. By photoelectric detection of the TV image intensity amplitudes down to 2  $\mu\text{m}$  could be measured. Preliminary results are presented.

Many investigations of the mechanical component of sound transmission of the middle ear have been carried out on human temporal bone preparations. Conclusions drawn from such experiments are uncertain as it is not known to what degree the behaviour of the mechanical system in living man corresponds in observations made from temporal bone preparations.

Experiments in vitro with human temporal bone provide no information about the effect of the muscles of the middle ear on the mechanics of movement.

The sizes of the amplitudes produced by the tympanic membrane and the ossicular chain are of importance in understanding the role this part of the mechanical system plays in the sensibility of the ear.

Wiltska (1935) measured the movement on the tympanic membrane at the threshold of hearing in one patient. This measurement was obtained by connecting the tympanic membrane to a thin rod leading from the movable coil in a dynamic loudspeaker. In this way he was able to cause the tympanic membrane to

record the rod's vibrations. The apparatus was calibrated by means of a microscope but the calibration was possible only for frequencies up to 270 Hz. His results for frequencies above 270 Hz were obtained by extrapolation and are therefore in our opinion unreliable.

Today we have in holography a method which may be used for measurements of the drum and part of the malleus in living man.

### HOLOGRAPHY

Holography is in principle a means of using laser light to create a unique photographic recording of the light reflected from an object without the use of a lens. This photographic recording is called a hologram. The hologram bears no resemblance to the image of the object but appears to be an unrecognizable pattern of stripes and whorls when examined under great magnification. However, when the hologram is illuminated by light from a laser the light is organized as by magic into a three-dimensional representation of the original object. The optical illusion is so realistic that it is impossible to decide whether the object is real or not without grasping for it physically.

An ordinary photographic image records the variations in intensity of light reflected from the object producing dark areas where less light is reflected and bright areas where more light is reflected. Holography however records not only the intensity of the object light but also its phase. In this connection the phase



Table I Number of mice per experimental condition

| Tracer       | Post injection sacrifice time (h) |   |   |   |    |
|--------------|-----------------------------------|---|---|---|----|
|              | 1                                 | 1 | 2 | 5 | 24 |
| Ferritin     | 6                                 | 4 | 3 | 4 | 7  |
| Iron dextran | 2                                 |   |   | 2 | 2  |

Many non-enzymatic electron-opaque tracers have been used to study capillary transport at the ultrastructural level. In particular ferritin has been successfully used to delineate certain aspects of transendothelial exchange in a variety of tissues (Farquhar et al 1961; Bruns & Palade 1968; Clementi & Palade 1969). There are many reasons why ferritin may be considered an ideal tracer (Bruns & Palade 1968) especially for strial capillaries. They include (1) ferritin (mol wt 460 000; mol diam 110 Å) is the size required for a probe molecule of the large pore transport system which is reportedly the only system in strial capillaries; (2) it is a biological substance similar in nature and in size to most plasma proteins; (3) high blood concentrations are well tolerated by experimental animals; (4) individual molecules can be identified with in ultrathin sections and therefore a direct measure of transport is possible; (5) ferritin is resistant to lysosomal hydrolases (Drysdale & Munro 1966; Coffey & DeDuve 1968) and therefore may be useful in pathologic conditions.

The advantage which the electron opaque tracer molecule ferritin may provide over enzymatic tracers warranted its evaluation. In addition because the iron dextran results reported by Yamamoto & Nakui (1964) are in conflict with other strial capillary permeability studies a reevaluation was judged necessary. Therefore it was the purpose of this study to evaluate strial capillary permeability with the electron-opaque tracer molecules ferritin and iron dextran.

## MATERIALS AND METHODS

### Tracer substances

Iron dextran (Imferon, Lakeside Labs Milwaukee Wis.) was dispersed in physiological saline with 0.5% phenol as a preservative and contained the equivalent of 50 mg elemental iron per ml.

A 10 ml stock solution of 100 mg of cadmium crystallized ferritin per ml of physiological saline was obtained (Nutritional Biochemicals, Cleveland Ohio). To remove the cadmium the solution was dialysed against 3.7% ethylenediamine tetraacetic acid (EDTA) in 0.2 M phosphate buffer pH 7.0 at 0–4°C for two changes (48 h each). Following two additional changes in buffer alone (24 h each) the ferritin solution was dialysed against physiological saline (24 h) and then passed through a 0.45 µm micrometer multipore filter into a sterile 10 ml injection vial.

### Animals

Young adult male mice BALB c/J (Jackson Labs Bar Harbor Maine) ranging in weight from 15 to 20 g and showing positive Preyer reflexes to finger snaps were used. The animals were screened for middle ear infection by direct examination during temporal bone removal.

### Methods

All animals received subcutaneous injections of sodium pentobarbital 2 mg/20 g body weight prior to injection of tracer. Anesthesia was reached within 15 min and lasted for 2–3 h for those animals allowed to recover. Under anesthesia either the jugular vein or the inferior vena cava was exposed and was injected with 1 ml per 100 g body weight of ferritin or iron dextran solution. The injection was delivered through a 30 gauge needle over a period of 1–2 min. Animals were sacrificed by decapitation 1/2, 1, 2, 5 or 24 h after injection. Twelve control animals received anesthetic injection only and were sacrificed immediately after induction. The number of

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O J Løkberg<sup>1</sup> K. Høgmoe<sup>2</sup> and T. Gundersen<sup>3</sup>

From the University of Trondheim, Norwegian Institute of Technology, Physics Department and <sup>2</sup>Department of Ear, Nose Throat Regional Hospital Trondheim, Norway

(Received March 1, 1979)

**Abstract.** The vibration patterns of the human eardrum in vivo have been recorded by time average electronic speckle pattern interferometry—ESPI. The necessary stability is achieved by shortening the exposure time of each TV frame. The amplitude—and phase—distribution was mapped across the drum by phase-modulation techniques which also could show the vibrations in slow motion. By photoelectric detection of the TV image intensity amplitudes down to 2  $\mu\text{m}$  could be measured. Preliminary results are presented.

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animals per experimental condition is noted in Table 1

Cochleae were fixed either in 1% osmium tetroxide or 2% glutaraldehyde in 0.2 M Cacodylate buffer pH 7.4. Within 1 min after decapitation the right temporal bone of each animal was excised, fractured open and immersed in about 5 ml of primary fixative. After 2 h of primary fixation at 0–4°C all cochleae were briefly washed in buffer. Those ears fixed in glutaraldehyde were then osmicated for an additional 2 h. However, from each cochlea of those animals injected with iron dextran pieces of stria were removed while in buffer and were not osmicated. At the completion of osmication the specimens were again briefly washed in buffer. In ferritin injected animals specimens were selected from basal, mid and apical portions of the cochlear duct. After the striae were microdissected free from the cochleae in 70% acetone dehydration was completed in graded acetone and the tissue embedded in Epon at 60°C for 24 h.

Ultra thin sections were cut on glass and diamond knives using either the Sorvall MT 1 ultramicrotome or an LKB Ultratome. Section interference colors ranged around silver. Sections were picked up on copper grids and most were examined unstained so that electron dense tracer particles could be unequivocally identified. Some sections were stained with lead citrate (Reynolds 1963). Contrast was significantly enhanced by the use of projector field diaphragms with the Zeiss 9S-2 EM.

## RESULTS

Those animals allowed to recover from anaesthesia had positive Preyer reflexes. None of the experimental ears showed evidence of middle ear infection upon direct examination using a dissecting microscope.

The ultrastructural appearance of the stria vascularis was typical of the fixation procedure employed (Merck et al. 1974; Santos Sacchi 1978a and b). One noteworthy difference between the two fixation procedures

was the decreased concentration of tracer particles within capillary lumina in primary osmium fixed specimens as compared to primary glutaraldehyde fixed tissues.

### *Iron dextran*

The plasma concentration of iron dextran decreased over time. In the 1/2 h specimens capillaries were observed which contained iron dextran only within endothelial pinocytotic vesicles (Fig. 1). However, more frequently and in all animals beyond 1/2 h up to 5 h the iron dextran appeared free as well as in vesicles within the cytoplasm of endothelia (Figs 2 and 3). In fact the tracer was occasionally observed within the nucleoplasm of some endothelia. By 24 h after injection however the iron dextran was absent from endothelia (Fig. 4). The passage of the tracer to the basal lamina was found to be almost nil since rarely were any electron dense particles found in the basal lamina at any time point after injection. No tracer was observed within stria cells.

### *Ferritin*

The appearance of ferritin within various spaces of the tissues studied was indicative of its passage from the circulatory system into the stria vascularis. The results are reported in terms of compartments within which ferritin was observed.

### *Capillary lumen*

From 1/2 to 24 h following injection ferritin was present within the capillary lumina of the stria in all areas studied. However the concentration of ferritin within the blood plasma varied with time. By 24 h after ferritin administration the plasma concentration was considerably lower than its initial level. Ferritin was never present within capillary lumina of control animals.

### *Endothelial tunic*

From the earliest to the latest time points after administration ferritin labeled pinocytotic

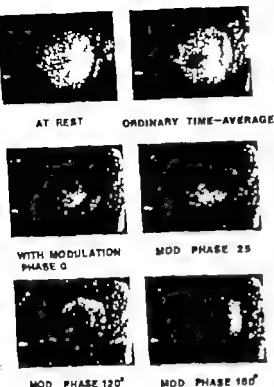


Fig. 4. ESP1 recordings of an ear-drum. Upper row: at rest and vibrating at 2400 Hz, 90 dB as recorded by ordinary time-average. The same sound pressure is used in the remaining pictures, but with the modulating mirror vibrating at 0.1  $\mu$ m and phase settings as indicated.

Between 3 and 4.5 kHz there is another peak and the explanation for this may be the increasing of the sound energy in the inner part of the extended malleus as shown by Wiener & Ross (1946). The measurement indicates that the size of the amplitudes of the malleus in living man is considerably greater (ten times?) than those found in temporal bone preparations. However we should bear in mind the difference in the SPL measurement procedures in the two cases.

Fig. 4 shows ESP1 interferograms photographed off the TV-monitor. The first is recorded with the membrane at rest; the others are recorded at 2400 Hz—90 dB SPL. No. 2 is recorded without reference wave modulation. The manubrium can be observed where the re-

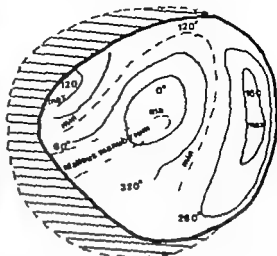


Fig. 5. Sketch indicating the behaviour of the eardrum in Fig. 4 (2400 Hz). The lines are isosplitude lines with max. and min. amplitudes as given. Phase values are given with numbers (hatched area: not observed part of the drum).

flectivity and the fringe contrast are better. In the other areas of the membrane the poor fringe contrast makes it difficult to observe any fringes.

The remaining four pictures are recorded with phase modulation and with different phase settings (0° 25° 120° 160°) which make the zero order fringe move about on the membrane. This is much better observed *live* on the TV monitor using a continuous phase shift than in the still photographs.

In Fig. 5 what is believed to be the vibratory behaviour of the tympanic membrane at 2400 Hz was sketched as an example. At this frequency the motion is complex with gross variations in vibration phase. In the other drum of Fig. 3 the movement was much more uniform with small phase variations. The amplitude of the manubrium tip was however quite equal.

## ZUSAMMENFASSUNG

Die Schwingungen des lebenden Menschentrommelfells sind registriert worden, mittels Durchsichtszern elektronischer Speckle-Interferometrie — ESP1. Die notwendige Stabilität wurde bei Verkürzung der Exponen-

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## THE ROLE OF MATRIX VESICLES IN THE PATHOGENESIS OF TYMPANOSCLEROSIS

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(Received January 31 1979)

**Abstract** In histological and electron microscopical study the authors investigated the pathogenesis of tympanosclerosis. Emphasis was placed on the role of extracellular lysosomes (matrix lysosomes). The function of such matrix lysosomes is connected with the two-stage degradation of the connective tissue. This system gets out of control in tympanosclerosis. Inflammatory and immunological processes are suggested to be superimposed on this lysosomal action.

Even today the pathogenesis of tympanosclerosis is unclear. When reviewing the literature most of the authors assume that some sort of chronic otitis media and a particular predisposition of the patient are a prerequisite (for refs. see Beck & Ebert 1964 Chang, 1969 Sorensen & True 1971). The clinical picture is well known and there is no need for further description.

Describing morphological changes during otitis media, alterations of the epithelium and of the submucosal layers must be regarded separately. During tympanosclerosis the superficial epithelium disappears and the submucosal connective tissue is profoundly modified. At the ultrastructural level the prominent changes are as follows: marked infiltration with lipid enclaves, remarkable changes of the collagenous fibres in structure and number and the presence of cellular debris and of calcium phosphate deposits (Chang, 1969). Preliminary studies have revealed that the cellular debris partially consists of matrix vesicles the lysosomal nature of which could be demonstrated in several connective tissue diseases (Riede & Staubesand 1977).

Considering the fact that lysosomes of the connective tissue cells contain hyaluronidases,  $\beta$ -glucuronidases,  $\beta$ - $\alpha$ -acetylglucosaminidases, proteases and cathepsins for proteoglycan degradation (Sledge 1968 Dingle 1969 Parakkal 1977) we were interested to study the formal pathogenesis of tympanosclerosis from this point of view.

### MATERIAL AND METHOD

#### *Histological preparation*

Tympanosclerotic plaques of the oval niche from four different patients (2  $\sigma$  and 2  $\eta$  aged 30-40 years) and tympanic mucosa of 2 patients with chronic purulent otitis media were fixed in 10% buffered formalin at room temperature for 24 h, embedded in paraffin wax and sectioned at a thickness of 5  $\mu$ m.

#### *Histochemical preparation and demonstration of oxytalan fibres*

The histological slides were stained as follows (Romeis 1968 Fullmer & Lilke 1958 Rannve 1963): Monopersulphate aldehyde fuchsin aldehyde fuchsin periodic acid-Schiff sequence haematoxylin-eosin Taenzer Unna's orcein and Azan's stain.

For the monopersulphate aldehyde fuchsin method the tissues were pre-oxidized in a 10% aqueous solution of potassium monopersulphate (Karoat Degussa, Hanau). The oxidizing properties of potassium monopersulphate are comparable to those of peracetic acid but

often empty on occasion ferritin could be visualized within the basal lamina either just in front of the opening or not far away (Fig 9). Nevertheless there were direct observations of ferritin within vesicles opened upon the luminal and tissue fronts (Figs 7, 8 and 9) as well as in those within the endothelial cytoplasm. Labeled vesicles within the endothelial cytoplasm were encountered more frequently. It cannot be ruled out that ferritin within open mouthed vesicles diffused out during fixation.

Another occasional phenomenon was the appearance of tubular invaginations from luminal and tissue surface membranes of endothelial cells (Fig 5). These structures were only encountered in thick portions of endothelia. Ferritin was found within some luminal invaginations but direct connections from luminal to tissue fronts were never observed.

There appeared to be a relation between the time after injection and the accumulation of ferritin within vacuoles of endothelia. At the earlier time points vacuoles containing ferritin in fairly large amounts were occasionally observed (Fig 8). Rarely large vesicles with high concentrations of ferritin were seen open to the plasma front as if in the process of loading (Fig. 5). With increasing time larger and denser cytoplasmic vacuoles were noted within which individual ferritin granules could be observed (Fig 11). Pinocytotic vesicles labeled with ferritin were seen fused with large ferritin containing vacuoles (Fig 8) or with each other (Fig 5). Multi vesicular bodies were also seen that in single membrane vacuoles within which were found labeled as well as unlabeled pinocytotic like vesicles (Fig 10).

Ferritin was neither seen in pinocytotic or other structures within endothelia of control animals nor was it observed free in the endothelial cytoplasm of experimental or control animals.

#### *Basal lamina and intercellular spaces*

The basal lamina was not heavily infiltrated with ferritin molecules at any time point after

injection. In the earlier specimens observed molecules were either absent or present at extremely low concentrations singly scattered about the perimeter of the capillary (Figs. 5 and 8). No accumulation of ferritin within the basal lamina at particular points around the capillary was noted save one instance. This occurred in a capillary of an animal sacrificed 1/2 h after injection (Fig 12). An apparent leak within a discrete portion of a single capillary allowed a relatively large quantity of ferritin to escape into the basal lamina. This was the only occurrence of this kind in all specimens studied and adjacent stria capillaries within the same section did not exhibit this profuse transendothelial passage of ferritin. This phenomenon was clearly not typical of normal stria capillary function.

In the 5 and 24 h specimens accumulation of ferritin appeared slightly greater around some capillaries than in earlier specimens. However this was not the rule since other capillaries at the same time periods showed little or no ferritin present within the basal lamina. The overall picture revealed the ferritin labeling of the basal lamina was variable at all time points studied and that labeling even at its greatest was scant.

*Fig 7* Large arrow indicates numerous labeled pinocytotic vesicles within endothelium. Smaller arrow indicates open-mouthed vesicle upon luminal front which appears to have ferritin molecules within neck portion of vesicle. 1 h post injection. Osmium fixed. Stained with lead citrate. Mid portion of cochlear duct.  $\times 97,325$ .

*Fig 8* Note numerous open-mouthed pinocytotic vesicles upon tissue front. Medium arrow indicates a ferritin labeled one. A ferritin molecule can be seen in the basal lamina (small arrow). Within the endothelial cell a pinocytotic vesicle can be seen fused with a large vacuole containing numerous ferritin molecules (large arrow). 5 h post injection. Glutaraldehyde and osmium II ed. Unstained. Basal portion of cochlear duct.  $\times 88,635$ .

*Fig 9* Large arrow indicates open-mouthed pinocytotic vesicle containing two ferritin molecules on luminal front. A ferritin molecule can be seen at the opening of pinocytotic vesicle at the tissue front (medium arrow). Small arrows indicate ferritin molecules within basal lamina. 5 h after injection. Osmium fixed. Unstained. Mid portion of cochlear duct.  $\times 97,470$ .



Fig. 3b Osmophilic degeneration of the ground substance (?) in between crystalline fibrils (ox) ( $\times 14\,000$ ). *Isaei*. Magnification,  $\times 23\,680$ .

peared isolated or clustered, calcified or uncalcified, extracellular or intracellular (Fig. 5a, b).

**Cells.** The dominant cell type belonged to the cell group of fibroblasts. Most of them were irregular-shaped and some were degenerating. Characteristics were: The increased osmophilic cytoplasm, the accumulation of cytoplasmatic vesicles in the peripheral cytoplasm and the increased number of cytoplasmatic processes. No giant cells (=macrophages) were observed.

Similar findings were seen to a minor degree in patients with chronic purulent otitis media, but never in healthy individuals.

## DISCUSSION

The prominent feature of the tympanosclerosis is a disturbance of the extracellular space in the submucosa. This means an alteration of the three main components, Elastin, collagen and proteoglycans. As there is an interaction between the composition of the extracellular space and the cellular components the pathogenesis of tympanosclerosis may be due either to a disturbance in the intercellular space or to a disturbance at the cellular level.

### Formal pathogenesis

With regard to the formal pathogenesis the most marked alteration is linked to the fibrous



Ferritin molecules within the intercellular spaces of the stria vascularis were not observed and presumably the size of the molecule limited its penetration into such spaces. Ferritin was not seen within the basal lamina of control animals.

### *Strial cells*

Ferritin was not seen within the marginal cells. However, large accumulations of electron dense particles closely resembling ferritin in size and shape were often found in specific cell processes of light cells (Fig. 6). In fact, the particles within these cells and the injected ferritin molecules appeared indistinguishable. The particles were found in high concentration free in the cytoplasm often accompanied by lysosomes containing the same particles. This phenomenon was found throughout all time periods studied and in control animals as well. Further description and analysis of these cells will be the subject of another report (Santos Sacchi and Marovitz in preparation). No pinocytotic uptake of injected ferritin by these light cells was seen, although light cells occasionally possessed open-mouthed vesicles on the basal lamina. Also, no spectacular accumulation of ferritin within vacuoles was ever observed in these cells. There were no apparent differences in cytoplasmic ferritin-like concentration between experimental and control animals.

## DISCUSSION

### *Iron dextran*

The iron dextran results were perplexing. Iron dextran was present within strial endothelial cytoplasm from 1/2 to at least 5 h but was apparently absent by 24 h. This did not seem to be a fixation artifact. It was concluded that the tracer initially entered the endothelia by means of pinocytotic uptake, since in some 1/2 h specimens and in the endothelia of a control animal (killed immediately after injection) iron dextran was present within vesicles and

absent within the cytoplasmic matrix. The occurrence of iron dextran within the cytoplasmic matrix of endothelia is difficult to explain since it is generally acknowledged that macromolecules have no route by which to enter the membrane protected cytoplasm. Although molecules the size of iron dextran are unable to pass through intact membranes, similar observations of free intracytoplasmic iron dextran following administration have been reported. Richter (1959) after injecting iron dextran intraperitoneally studied the fate of that substance within liver and spleen. After 1 h he found accumulations of iron dextran within macrophages and sinusoidal endothelia of both organs. There were non-membrane bound aggregates of iron dextran within the cytoplasmic matrices as well as numerous free particles. This is similar to the results of the present study. However, in Richter's study by the fourth hour following injection aggregates were enclosed by limiting membranes. Further, iron dextran was discernible within the cells up to 6 days after administration. In this regard Richter's results differ from the present study because iron dextran was absent from 24 h. However, it should be recalled that in the only previous study of iron dextran permeability in strial capillaries (Yamamoto & Nakai, 1964) the molecules remained within endothelia, reportedly within vacuoles for 5 h but by 24 h and beyond iron dextran was absent from endothelia. It seems that strial endothelia have an ability to remove or possibly metabolize iron dextran so that by 24 h very little or none remains observable. Yamamoto and Nakai reported no transendothelial passage of iron dextran into the stria. Although this appears to be the case for the present study as well, the possibility exists that passage of some degraded form of the original molecule occurred which could not be visualized electronmicroscopically. Richter's experiment suggests that mouse cells may be able to metabolize the partially depolymerized dextran sufficiently to abolish the colloidal stability of the iron dextran preparation.

ment and to an alteration of the proteoglycans concerned with fibrillogenesis (Tyberg 1974).

2. There is calcification of the connective tissue. Type I matrix vesicles contain alkaline phosphatase which cleaves inorganic pyrophosphate inhibitors of calcification as uncouplers in the cell membrane and contain ATPases for the active transport of calcium into Type II matrix vesicles (Anderson 1976). First crystallization of calcium apatite takes place in these matrix vesicles Type II (Höbling et al. 1976).

Considering the pathogenesis of tympanosclerosis from the clinical point of view the disease may be affected positively by anti-necrotic agents with lysosomal stabilizing effects such as cortisone, phenylbutazone, anti-histamines (Weissmann 1969) and Flavichromin (Schole et al. 1978; Jonas & Riede 1979). However this statement has no clinical relevance as long as there is no possibility of detecting the initial phase of tympanosclerosis. This is the aim of further experimental and clinical investigations.

## ZUSAMMENFASSUNG

In einer histochemischen und ultrastrukturellen Arbeit werden die pathologischen Bindegewebsveränderungen bei der Tympanosklerose analysiert. Die Autoren entwickeln ein Konzept zur formalen Pathogenese dieser Erkrankung. Eine wichtige Rolle wird den extrazellulär liegenden Lysosomen (=Matrixvesikel) zugesprochen, die am „2. Schritt-Abbau“ des Bindegewebes entscheidend mitbeteiligt sind. Für die Tympanosklerose postulieren die Autoren eine Dysfunktion dieses Systems, verbunden mit einer verstärkten lysosomalen Aktivität. Dieser autophagolytischen Reaktion scheinen sich entzündliche und mechanologische Prozesse anzuschließen.

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Of course diffuse iron in ionic form or bound in low concentrations to carrier substances cannot be seen (Muir & Goldberg 1961)

Clearly then the use of iron dextran as an electron opaque tracer molecule for stria capillary transport is obviated by the possibly unique treatment it receives by stria endothelia which apparently results in the intra cytoplasmic sequestration and eventual removal of iron dextran

### *Ferritin*

Although ferritin transport across stria capillaries had not been evaluated prior to the present study HRP transport had been examined. The transport of the enzyme through stria vessels is very rapid. Within a few min after HRP injection the basal lamina and intercellular spaces are densely stained with reaction product (Duvall et al. 1971; Winther 1971). These results are more similar to those obtained with muscle capillaries than to those obtained with CNS capillaries except that intercellular cleft transport of HRP is reportedly absent in stria capillaries. However the results of the present study which suggest minimal capillary transport indicate that the permeability function of the stria capillaries is more similar to that of the CNS vessels. Thus even by 24 h post injection transport of ferritin across endothelia is scanty as determined by basal lamina labeling. Although transport of lipid insoluble substances certainly increases as molecular weight decreases the evidence evaluated by Renkin (1964) suggests that transport rates of molecules in excess of about 60 000 Daltons remains constant. If the micro-pinocytotic transport system is the sole means of transendothelial passage of lipid insoluble material within the stria, then the passage of HRP (whose molecular weight is close to Renkin's cut off size) should not be drastically different from that of ferritin. This may especially be true if as some researchers believe (Balint & Nagy 1971) HRP binds to B-globulin and IgG within plasma thereby increasing

the particle size and bringing it closer to the size of ferritin. Thus by size comparison it is difficult to explain the similarity of HRP results and the dissimilarity of ferritin results between stria and muscle capillaries. Certainly the very small number of labeled stria capillary pinocytotic vesicles could account for the smaller amount of ferritin transport in stria as compared to muscle capillaries. Moreover the dissimilarity of transport suggests that some other factor may play an important role in the rapid transport of HRP across stria endothelia. It is here that the established ototoxicity of HRP may come to bear (Ross et al. 1977). Perhaps the transport of HRP is rapid in stria capillaries because the HRP itself alters stria capillary permeability. Such a hypothesis would explain the similarity of HRP results in muscle and stria capillaries and would not conflict with the ferritin results.

Research on the permeability of continuous type capillaries in other tissues also leads to the conclusion that the stria HRP results are unusual i.e. in the light of this study's findings with ferritin. Raviola & Karnovsky (1972) evaluated thymic capillary transport using a variety of enzymatic tracers including HRP. They also used the tracer molecule ferritin so that it is possible to compare the permeabilities of both HRP and ferritin in the same capillary system. One must note however that the thymus contains both continuous and fenestrated capillaries. For the present purpose only the continuous capillaries permeability functions are of significance since these capillaries are structurally comparable to stria capillaries. Interestingly these continuous capillaries located within the cortical areas of the thymus present a blood-thymic barrier. As in the stria and CNS circumferential tight junctions halt extravascular leakage through endothelial intercellular clefts. At all time periods from 1-24 h after ferritin injection minimal amounts of the electron opaque tracer had traversed the thymic capillary endothelia. Labeling of pinocytotic vesicles was variable but represented a very

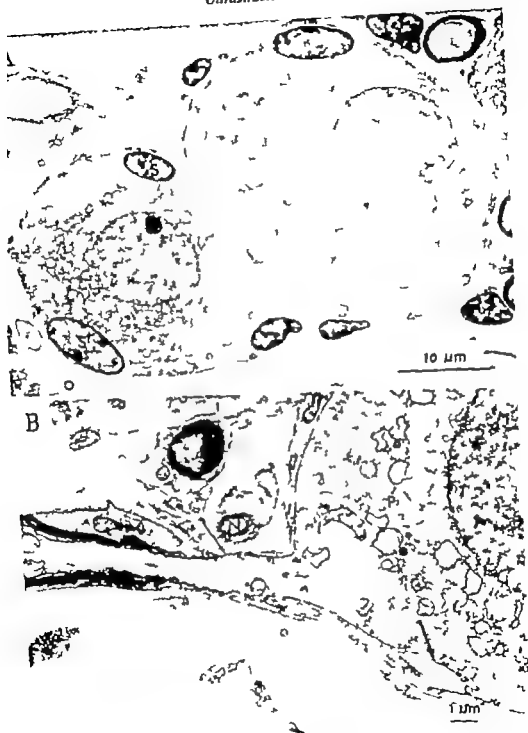


Fig. 2 (A) An electron micrograph of the typical large unmyelinated neuron. The lower perikaryon is surrounded by two satellite cells (S) and shows long axonal process. (B) A dendritic process of the large unmyelinated neuron showing myelination a short distance from the perikaryon (P). Note the lack of

Schwann cell covering at the node of Ranvier (small arrow) and nearby nerve fiber (N). The light elongated structures on the lower right corner (large arrow) is either another process of the satellite cell or an evagination of the perikaryon.

between control and experimental animals in regard to concentration of intracytoplasmic ferritin-like particles. Nevertheless within the cytoplasm of liver cells there is *de novo* apoferritin production within a few h following administration subsequently iron sequestration by apoferritin occurs producing ferritin (Drysdale & Munro 1966). In the present study since the injected tracer contains iron the *de novo* production of endogenous ferritin by experimental strial light cells might occur if indeed they phagocytized transported exogenous ferritin. The problem is made more difficult because the horse spleen ferritin tracer is very resistant to lysosomal breakdown (Coffey & DeDuve 1968). Thus although ferritin may accumulate within lysosomes elemental iron may not be able to enter the cytoplasm because of the hydrolytic resistance of the protein. On the other hand there is some evidence that the iron may enter and leave the apoferritin shell without protein disintegration (Mazur & Carleton 1963). In this regard it is noteworthy that in other permeability studies using ferritin as a tracer (Bruns & Palade 1968; Raviola & Karnovsky 1972) after 24 h perivascular macrophages did exhibit intracytoplasmic ferritin granules. Presumably they were endogenously produced after the phagocytes accumulated the ferritin tracer within lysosomes. In the present study since numerous lysosomes containing ferritin were found in control animals a normal turnover mechanism may be deduced. However it cannot be totally dismissed that the select light cells in experimental animals also contain exogenously injected ferritin within lysosomes. Finally it should be noted that although light cells often exhibited open-mouthed vesicles upon the basal lamina no ferritin labeling of these vesicles in experimental animals was found. Certainly the scanty accumulation of ferritin within the basal lamina offered little probability of finding ferritin within such open-mouthed vesicles.

The central nervous system of mammals appears to require an unusually stable internal

environment in order to function effectively (Katzman 1972). Clearly the blood-brain barrier affords such an environment in a like manner thymic and myenteric plexus blood-tissue barriers serve their own physiological purposes. The finding that the transport of ferritin across strial endothelia is scanty may indicate that the internal milieu of the strium must be carefully regulated. The probable existence of strial phagocytic cells may also be important in this regard. Normal strial function may very well depend upon an intact blood strial barrier.

The ferritin labeling technique used in this study of normal animals may be useful in determining altered strial capillary transport possibly associated with various auditory pathologies. It is known that under pathologic conditions of inflammation extravascular leakage of high protein content fluid occurs (Florey 1964). Indeed this is known to occur in brain tissue and involve a breakdown of the blood brain barrier (Katzman 1972). Certainly under some deleterious influences strial tissue becomes edematous as in response to ethacrynic acid or noise exposure. In the present study only one instance of abundant ferritin accumulation within the basal lamina was observed. Since adjacent capillaries within the same section and all other capillaries did not exhibit such abundant transport the phenomenon was judged atypical of strial capillary transport. However this occurrence indicates that the tracer molecule ferritin may be useful in determining altered strial transport. Ferritin is highly resistant to lysosomal hydrolases (Coffey & DeDuve 1968). Therefore if large amounts did traverse the endothelial tunic they would no doubt be detected within some "compartment" of strial tissue assuming of course that the tight junctional seal delimiting the strial vasculature remains intact. However the possibility of light cell incorporation of exogenous tracer and the difficulty in differentiating between endogenous and exogenous electron dense particles should be kept cognizant.

a few thin layers of the sheath or some loose layers of myelin were shown at some parts of the perikarya. The cytoplasm of the large unmyelinated neurons was rich with mitochondria in various forms curvature and length. Numerous short and elongated rough endoplasmic reticulum as well as ribosomes in rosette form were scattered throughout. Nissl bodies were randomly located and the Golgi network was not conspicuous. Round lysosomal granules were a common finding and lipofuscin masses were observed in specimens from individuals aged 7 years and older though their number and size did not increase in direct proportion to age. Intracellular neurofilaments were sparse. Toward the central process (axon) the cell organelles decreased and the neurofilaments and homogeneous substance increased. The axon hillocks were large and often long. Their processes became myelinated at a distance of about 4 to 38  $\mu\text{m}$  from the perikarya. Only in one instance was a thick myelin formation observed near the axon hillock. Toward the peripheral process (dendrite) there was a decrease in cell organelles and there were considerably fewer neurofilaments than on the axonal side. The site of origin of the dendrite was often difficult to determine because of the small size. Unless the sections were examined at close intervals one would get the impression of a unipolar neuron or even a pathological specimen with atrophy of the dendrites. The dendritic process also became myelinated at a distance of 5 to 26  $\mu\text{m}$  from the perikaryon (Fig. 2B) but myelination at the border of the perikaryon was not observed. The diameter of the dendritic process near the perikaryon was about one-fourth to three-fourths the size of the axon. It came out straight or twisted, sometimes not directly opposite to the axon but close to it. As yet, no branching of these processes was seen at the proximity of the perikarya. No multipolar neurons were observed among the large neurons.

The large myelinated neurons were few in

number (Fig. 3A, B). Their size was similar to that of the unmyelinated type. They were located randomly within the neuronal masses throughout the cochlea and they were often seen in the aged individual. The highest count was made in a specimen from a 75-year-old 11 out of 39 or 28%. In the youngest individual (9 months) no myelinated neurons were found among the 94 neurons counted. The average of each individual percentage of specimens between the ages of 9 months and 42 years showed only 1% myelinated neurons. Between the ages of 65 and 92, the average increased to 19% excluding the Meniere's specimen. If the Meniere's case (age 59) which appeared to be normal, were included the average number of myelinated neurons became even smaller 5.8%. All cell counts were made from composite electron micrographs however the samples were mostly taken from the mid-portion of Rosenthal's canal as the bony parts along with some neurons had to be trimmed away for sectioning. The number of myelin layers varied somewhere in the order of 4 to 17 (Fig. 3B) and were often not uniform from one part to another part of the neuron. The neurons retaining a few sheaths of satellite cells or the partly myelinated types were classified as unmyelinated, since they would not be positively identifiable under low magnification or under the phase contrast microscope. Myelin sheath was observed in both loose and compact forms though the loose form was most common. The cytoplasmic characteristics of both myelinated and unmyelinated large neurons were similar.

The small spiral ganglion cell was 8 to 14  $\mu\text{m}$  in diameter and 15 to 21  $\mu\text{m}$  in length. There were both unmyelinated (Fig. 4A, B) and myelinated (Fig. 5A, B) types. Some small unmyelinated neurons found among the large neuron masses showed a cytological profile similar to the large ones. Another distinctly different type of small neuron was bipolar and a few cells might have been

between control and experimental animals in regard to concentration of intracytoplasmic ferritin like particles. Nevertheless within the cytoplasm of liver cells there is *de novo* apoferritin production within a few h following administration subsequently iron sequestration by apoferritin occurs producing ferritin (Drysdale & Munro 1966). In the present study since the injected tracer contains iron the *de novo* production of endogenous ferritin by experimental strial light cells might occur if indeed they phagocytized transported exogenous ferritin. The problem is made more difficult because the horse spleen ferritin tracer is very resistant to lysosomal breakdown (Coffey & DeDuve 1968). Thus although ferritin may accumulate within lysosomes elemental iron may not be able to enter the cytoplasm because of the hydrolytic resistance of the protein. On the other hand there is some evidence that the iron may enter and leave the apoferritin shell without protein disintegration (Mazur & Carleton 1963). In this regard it is noteworthy that in other permeability studies using ferritin as a tracer (Bruns & Palade 1968; Raviola & Karnovsky 1972) after 24 h perivascular macrophages did exhibit intracytoplasmic ferritin granules. Presumably they were endogenously produced after the phagocytes accumulated the ferritin tracer within lysosomes. In the present study since numerous lysosomes containing ferritin were found in control animals a normal turnover mechanism may be deduced. However it cannot be totally dismissed that the select light cells in experimental animals also contain exogenously injected ferritin within lysosomes. Finally it should be noted that although light cells often exhibited open mouthed vesicles upon the basal lamina no ferritin labeling of these vesicles in experimental animals was found. Certainly the scanty accumulation of ferritin within the basal lamina offered little probability of finding ferritin within such open-mouthed vesicles.

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laries and connective tissue as well as integrity of their neuronal processes: environment, the function of the neurons, their fiber destinations may determine the biological characteristics of satellite cells or Schwann cells.

In the cat large unmyelinated neurons are not normally present and were only seen in pathological specimens in which the myelinated type was converted into the unmyelinated type (Spoendlin 1974). Although large unmyelinated neurons were often observed in the hydropic inner ear of the guinea pig (Kimura unpublished data) degenerative changes were not always associated with the loss of myelin sheath. We observed degenerated neurons with intact myelin sheaths though the myelin was a loose form. Thus, it appears that neurons degenerate with or without the loss of myelin sheath. When we initially saw a majority of unmyelinated spiral ganglion cells in human Meniere's disease specimens we thought that these neurons might be one of the causes of the hearing loss (Kimura et al. 1976). However, since all other human spiral ganglion cells from young and old individuals were unmyelinated, such a notion was discarded.

Spoendlin (1971, 1972) suggested that the small unmyelinated neurons innervate the outer hair cells and Ross (1973) has suggested that the origin of these neurons is parasympathetic. The population of these small unmyelinated neurons is small: 5% in the cat (Spoendlin 1971), 10% in the guinea pig (Kellerhals et al. 1967) and 7 to 8% in the rat (Ross & Buskel 1973). Although our sample is rather small (due to our study being conducted at the ultrastructural level) the population was found to be about 6%. The morphology of these small neurons differs somewhat among different species. For example, in the cat they are unmyelinated and show either unipolar or bipolar processes. Their cytoplasm is filamentous. The nuclei are lobulated, eccentrically lo-

cated, and the nucleolus is not very conspicuous. In the rat they are also unmyelinated but the cell processes are multipolar. The cytoplasm contains abundant neurofilaments and cored vesicles, the latter of which are thought to be one of the characteristics of parasympathetic neurons. In our human material the small neurons are both myelinated and unmyelinated with either bipolar and/or multipolar processes. The exact number of cell processes could not be determined due to the wide gaps or jumps from one section to the next in the area of particular interest. The cytoplasm was filamentous but dense-cored vesicles were not observed. The nuclei were round and more or less centrally located. Their nucleoli differed from those of the large neurons and showed loose and irregular forms. The only common morphological traits among the different species are that these neurons are small and the cytoplasm contains numerous neurofilaments. Thus, it is difficult to determine whether these neurons observed in each species correspond to each other functionally or whether there are other types of small neurons present which have not yet been identified. From the present study it cannot be concluded whether these neurons innervate the organ of Corti or terminate somewhere in the modiolus or in the osseous spiral lamina. Nonetheless, the neurons differ significantly in their cytoplasm from the majority of large neurons.

Although it is not clear whether the function of small neurons is auditory or autonomic, the large neurons forming the majority of the population are presumed to reach the organ of Corti based on studies by Retzius (1895), Held (1926), Lorente de Nó (1937) and Polyak et al. (1946). When the nerve fibers were counted at the habenula and Corti's tunnel in the human, at least 78 to 85% of the nerve fibers went to the inner hair cell area and a maximum of 15 to 22% of the fibers went toward the outer hair cells (Kimura, 1975). The percentages representing



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Grason-Stadler 1720 large errors would accrue.

Consequently the circuit shown diagrammatically in Fig. 1 was developed which performs a vector subtraction of the admittance of the ear canal. This is free of errors for all frequencies as long as the simple lumped-admittance representation of the ear canal is appropriate.

In the circuit, a signal at the probe tone frequency is derived from the probe tone oscillator and can be adjusted manually in magnitude and phase. The resultant signal is then subtracted from the AVC output signal by the differential amplifier shown in the figure. The magnitude and phase controls are adjusted with the ear canal pressure at 200 mm of water above the middle-ear pressure when the eardrum admittance is near zero to obtain zero output from the differential amplifier. Thus the ear canal admittance is cancelled out. Any subsequent measurements automatically give the admittance modulus at the eardrum based on the assumption that the eardrum admittance is zero when there is a static pressure differential across the eardrum of 200 mm of water.

The output of the differential amplifier is rectified and smoothed and there is provision to offset the output to remove any pedestal voltage and allow a high degree of subsequent amplification for sensitive recording of acoustic reflexes.

The circuit thus provides an output which is proportional to the admittance modulus at the eardrum and has a transient response which is essentially that of the AVC. The latter has been increased by a factor of two by halving a capacitance in the AVC circuit and Fig. 2 illustrates the step response obtained by using a method similar to that of Popelka & Dubno (1978). Admittance modulus, conductance and susceptance outputs are compared in the figure. This illustrates

that the admittance modulus output does not exhibit the overshoot which is apparent for conductance and susceptance outputs. The rise time of the admittance modulus is approximately 40 ms and this is adequate to record acoustic reflexes accurately in humans which Dallos (1964) has shown to have rise times greater than 120 ms.

Use of this circuit makes it possible to obtain acoustic reflex waveforms in terms of an absolute linear measure the admittance modulus in the plane of the eardrum at probe tone frequencies of 220 and 660 Hz effectively uncontaminated by the temporal response characteristics of the instrumentation. The accuracy of the apparatus has been extensively verified by comparing its steady-state output with the conductance and susceptance outputs and the complete admittance measurement system has proved to be reliable during four years of research work.

## ZUSAMMENFASSUNG

Stapediusreflexmessungen, die mit dem Grason-Stadler 1720 Oto-Admittanzgerät durchgeführt werden, leiden unter einer meßfehlerverursachten Überacknowledging, die auf das Elmschwingungsverhalten des Meßgerätes zurückzuführen ist. Um dieses Problem zu lösen, wurde eine besondere Schaltungsanordnung entwickelt, die es ermöglicht, die statische Charakteristik des Stapediusreflexes genau und ohne dessen meßfehlerbehafteten Fehler aufzuzeichnen.

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Table II Table illustrating the hair cell loss in animals belonging to groups A and B

Injection of test solution was performed in the right ear. Hair cell loss below 1% was regarded as normal and was not noted in this table. In the cytochromeoxidase, counting of the most apical row (18–19 rows from the round window) was excluded because of the great variation in hair cells present during normal conditions.

OHC, outer hair cells; IHC, inner hair cells

| normal  | Hair cell loss (%) in various coils (right ear) |     |     |     |     |     |
|---------|---|-----|-----|-----|-----|-----|
|         | 1st   |     | 2nd |     | 3rd |     |
|         | OHC   | IHC | OHC | IHC | OHC | IHC |
| Group A |   |     |     |     |     |     |
|         |   |     |     |     | 3   |     |
|         |   |     |     |     | 7.6 |     |
|         |   |     | 12  | 5   | 8   | 5   |
| Group B |   |     |     |     |     |     |
|         |   |     |     |     | 6   |     |
|         |   |     |     |     |     | 7   |

The left ear was normal with regard to hair cell count.

was performed as a double-blind testing model. After finishing the experiments the code for each solution was received and the various components were identified (Table I).

Each solution was administered only once into the right bulla tympanica via a fine needle through the tympanic membrane under an operating microscope after general anaesthesia of the animal using ether narcosis via an open mask.

One animal from each group was sacrificed for morphological analysis of the inner ear 24 hours post-injection whilst the remaining animals were taken for morphology 5 weeks after the injection. The principles for morphological preparation of the membranous labyrinth (in situ immersion) have been described by Arnika & Lundquist (1979) using 1% osmic tetroxide as a fixative. All cochleae were prepared for cytochromeoxidase analysis, a technique described in detail by Ylikoski (1974). Spoendlin & Brun (1974) and others. According to the cytochromeoxidase study selected parts were thereafter chosen for light microscopic sectioning, as also were all cristae ampullares and macula utriculi (staining: toluidine blue).

Five labyrinths from group A and six from group B were decalcified in a solution of

EDTA (4.13% pH 7.0–7.4) prior to further handling after fixation of the membranous labyrinth.

## RESULTS

### I Clinical Observations

A few minutes after injection with solution A or B the guinea pigs already awake from the anaesthesia, revealed signs of marked vestibular dysfunction. Each animal showed a waltzing behaviour and nystagmus grade III with the fast component of eye movement towards the right side. These symptoms abated gradually after a couple of hours but could still be provoked 24–48 hours later. Guinea pigs injected with solution C remained normal.

On investigation 5 weeks post-injection animals in groups A and B had either an impaired Preyer's reflex on the right side or else this was completely lacking (most animals).

### II Middle Ear Cavity (Figs 1–4)

#### Solutions A and B

Animals investigated 24 hours after the intratympanic injection showed necrotic mucosal membranes and patchy haemorrhage in the middle ear.

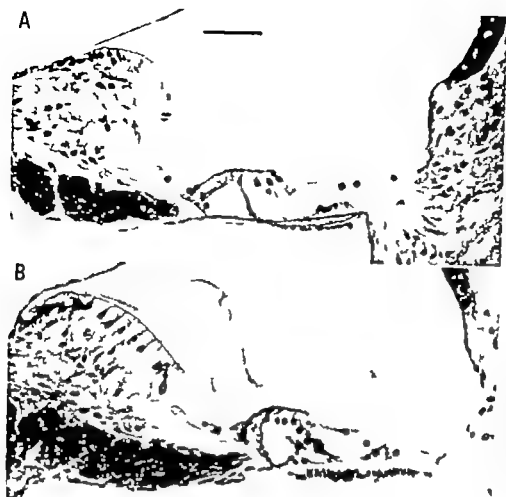


Fig 1 Two-micron thick araldite sections of the adult cochlea, stained with toluidine blue. Bar represents 50 microns. (A) C57Bl untreated control. (B) C57Bl mouse which has been treated with thioacetamide throughout its development. The tectorial membrane is grossly distorted

mutant genes are autosomal recessive but *Varitint Waddler* (Va) is autosomal semi dominant and heterozygous (Va/+) animals were used in this study. All three groups of mice are deaf. *Jerker* and *Varitint Waddler* mice also behavioural abnormalities and *Varitint Waddler* mutants have distinctive coat colours (Cloudman & Bunker 1945 Gröneberg et al 1941 Deol & Kocher 1958).

The mice were killed approximately two months after birth their outer capsules were removed and the cochleas cleared of surrounding tissue and placed in fresh distilled water. The tectorial membrane was separated from the organ of Corti and a finely drawn out glass pipette was used to transfer the pieces of membrane to several drops of fresh distilled water in turn until they were thoroughly rinsed and free of debris. The pieces were then examined under phase contrast and stored at  $-20^{\circ}\text{C}$  until required. Each sample

was collected either from the two cochleas of one animal or from four cochleas of two litter mates.

The time taken between killing the animal and freezing the specimen was noted in each case and varied between 40 minutes and two hours. This was not likely to lead to tissue damage as the tectorial membrane is an acellular matrix containing no lysosomes. However in order to test this possibility two samples were left at room temperature for 6 and 24 hours respectively before treatment. No significant differences were found in the banding pattern or intensity of staining of gels between these samples and those frozen only 40 minutes after death.

As the tectorial membrane is normally insoluble sodium dodecyl sulphate (SDS) was used to dissociate the material into its protein subunits before subjecting the sample to electrophoresis on disc polyacrylamide gels. The

When the bulla tympanica was explored after 5 weeks the middle ear in most animals was extensively filled with superimposed newly formed bone. The ossicles and the cochlea were encapsulated by this bone (Figs 3 & 4). On dissection however the ossicles could be freed fairly easily in contrast to the cochlear bone wall which was in continuous contact with the newly formed bone.

When dividing the auditory and vestibular parts of the inner ear the gross structure of the membranous labyrinth was unaffected and no growth of bone or fibrous tissue was identified in the vestibulum immediately inside the stapedial footplate. The round window area in most cases (9/14) was covered with superimposed newly formed bone.

#### *Solution B*

The bulla tympanica was completely normal

### III The Inner Ear

#### *Solution A*

The animal investigated 24 hours after the injection revealed a normal hair cell (HC) pattern at all levels in the cochlea, i.e. less than 1% HC loss, as also did 5/9 guinea pigs analysed 5 weeks after the injection. One animal died before morphological investigation was performed. Three remaining guinea pigs revealed a low percentage of HC loss (outer and inner hair cells, OHC and IHC respectively) mainly in the apical coils (Table II).

Fig. 1. Left middle ear cavity from animal no. 9 in group A showing normal conditions (non-injected side). The ossicles are removed. Arrow: round window membrane. Cf. Fig. 4.

Fig. 2. Right cochlea from animal no. 2 (injected side) in group C showing normal gross morphology. A part of the bone covering the different coils has been removed. Arrow: round window.

Fig. 3. Right bulla tympanica from guinea pig no. 4 in group III. Extensive formation of new bone in the middle ear cavity. A part of the bone covering the cochlea has been removed.

Fig. 4. Right middle ear cavity from animal no. 9 in group A. Cf. Fig. 1. The cochlear wall is thickened due to superimposed bone. The ossicles cannot be identified. Arrow: indicating the round window region.

Light microscopic sections through the vestibular organs showed normal morphology in all but one animal (no. 8). All three cristae ampullares of this animal (on the injected side) showed degenerating hair cells of both types on the top of the cristae.

#### *Solution B*

In this group of guinea pigs all but one (no. 3) showed a normal morphology in the cochlea and cristae ampullares. The aberrant animal had a loss of 7% OHC in the 4th coil and 6% OHC missing in the 3rd turn but an otherwise normal hair cell count (Table II).

#### *Solution C*

All 10 animals revealed a normal morphology in the inner ear organs.

### COMMENT

It is difficult to establish an ototoxic effect in the present study. No signs of acute damage to cochlear hair cells and those of the vestibular organs occurred except in one animal. In the cochlea degenerated hair cells had been replaced by phalangeal scarring.

In only one guinea pig was there an obvious increased hair cell loss indicative of a possible toxic effect of the drug, since the inner ear on the non-injected side showed normal conditions. The other animals with an outer hair cell loss below 10% in the apical turns might—according to the literature—be within the normal range of variation (Federspiel 1972, Ulehlová, 1973, Anniko & Sarkady 1978, Ulehlová & Voldrich 1978). The present control group of guinea pigs showed however less than 1% outer hair cell degeneration throughout the cochlea (except for the most distal part, 18–19 mm from the round window) thereby indicative of an ototoxic effect of the solutions A and B.

Whether or not the hair cell loss represents true ototoxic damage or is connected with or dependent upon the bone reaction in the bulla tympanica, e.g. disturbed microcirculation

parison of relative mobilities (Weber & Osborn 1969). The proteins used were RNA polymerase (165 000 155 000 39 000)  $\beta$ -galactosidase (135 000) phosphorylase a (92 500) bovine serum albumin (69 000) pyruvate kinase (60 000) catalase (57 500) phosphoglycerate kinase (47 000) aldolase (40 000) and glyceraldehyde 3-phosphate dehydrogenase (37 000).

Celloidin sections of cochleas from mice of each type were prepared by standard methods and these confirmed that the histological appearance of the tectorial membranes used in the present series was as described in previous reports (Deol 1954 1973 Deol & Kocher 1958).

An additional five samples of normal membranes from C57Bl mice were subjected to electrophoresis fixed and stained by the PAS technique (Fairbanks et al. 1971). These gels were later stained with Coomassie blue to confirm the presence of the tectorial membrane proteins. Gels containing known amounts of transferrin ovalbumin or ovomucoid were also stained using the PAS procedure to serve as controls.

## RESULTS

Isolated tectorial membranes from normal animals were virtually transparent until they were examined under phase contrast and some of the pieces became highly coiled in the distilled water. As they were drawn through the narrow pipette tip the membranes were deformed but they seemed to be springy and quickly returned to their original shape when released.

Membranes from thiouracil treated mice were generally more opaque with little tendency to form tight coils as in normal membranes. When they were deformed by being constricted in a pipette they took a long time to return to their original shape and some showed no signs of returning at all within the duration of the dissection. Their behaviour was more plastic than elastic and some behaved almost like formalin-fixed membranes.

Isolated membranes from the three mutants appeared to be indistinguishable from normal tectorial membranes.

The banding pattern of normal tectorial membranes from the four different genetic backgrounds is shown in Fig. 2a. The major band had a molecular weight of around 140 000. There were at least 3 components of higher molecular weight: two sharp bands at 155 000 and 165 000 and a more diffuse band at 180 000. Further towards the anode a pair of bands at 105 000 and 95 000 could usually be identified and between 62 000 and 77 000 there was a wide area of staining which could often be resolved into at least five different bands. The bands close to the dye front were rather variable as can be seen in Fig. 2a. Up to 2 bands could be distinguished by eye in any one gel.

No significant differences could be detected in the banding pattern between abnormal and control tectorial membranes as shown in Fig. 2b.

The transferrin ovalbumin and ovomucoid bands stained bright pink in gels stained by the PAS method. Ovomucoid contains 25% carbohydrate (Pearse 1968) so as about 20  $\mu$ g of this glycoprotein was loaded there should have been around 5  $\mu$ g of carbohydrate present. Transferrin contains 6% carbohydrate but only 4% is Schiff positive (Simpson et al. 1974). Again 20  $\mu$ g was loaded so the staining was readily detectable when only 0.8  $\mu$ g of reactive carbohydrate was present. Ovalbumin contains only 4% carbohydrate and part of this is resistant to periodate oxidation (Eckhardt et al. 1976) so a positive reaction was found in this case with less than 0.8  $\mu$ g of suitable carbohydrate.

No trace of staining was found for the tectorial membrane samples although the usual protein bands were observed when the gel was stained with Coomassie blue. The amount of material in these samples as judged by the intensity of Coomassie blue staining was probably of the order of 5–10  $\mu$ g per sample collected from two animals. Assuming a value of

## ARTIFACTS IN EIGHTH CRANIAL NERVE BIOPSY

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**Abstract** The occurrence of artifactual morphological changes was investigated in human eighth nerve biopsies and corresponding changes were brought about in biopsies from rats by mechanical handling before fixation. At the very site of compression by a pair of forceps the biopsy stained lighter than in its immediate surroundings. Electron microscopy showed denuded and fragmented axons among vesicular debris in the area. Farther away the myelin coats were greatly thickened, often to the degree that the axonal canal could no longer be identified. The myelin lamellae showed separation and were occasionally seen as convoluted forms. Also variations in the diameters of the myelin fibres and ruptures of the myelin coats could be produced after mechanical handling.

Great caution should be taken to avoid artifacts in biopsies of nerve tissue. This is a good general rule accepted by most research workers in the field of neuropathology. It is quite obvious, however, that this cannot always be avoided. In experimental work perfusion fixation gives superb preservation but biopsies from man may be subject to artifacts because of handling or autolysis before fixation. Under certain circumstances the available biopsies are necessarily very small and in these artifactual changes are most imminent. Our project to study the neuropathology of the human eighth nerve and the inner ear (Ylikoski et al. 1978a, Ylikoski et al. 1978b, Palva et al. 1978) included a series of experiments relative to artifacts which may arise in these structures as a result of handling. The findings are reported in this study.

### MATERIAL AND METHODS

For this study 150 biopsies of eighth nerve were available. The biopsies were carefully

checked for sites of compression caused by a pair of forceps. Sites of compression were studied by light and electron microscopy. In two cases the biopsy was cut in two and one half was artificially crushed several times with small forceps in order to create numerous artifacts. These samples were compared with the other biopsies. In addition three 1-year-old male rats were anesthetized with a barbiturate preparation and the eighth and fifth nerve uncovered by the occipital route. Biopsies were taken on the right side with a biopsy forceps whereupon the whole body of the animal was perfused with 3% glutaraldehyde in 1% phosphate buffer through a cannula inserted into the aorta. The fixative was allowed to act for 15 min whereafter biopsies were taken from the left side. The biopsies were fixed further in the same fixative at +4°C for 24 h and thereafter transferred into sucrose. They were postfixed in 1% OsO<sub>4</sub> in 1% phosphate buffer and embedded in Epon. Sections were cut with glass knives for light and electron microscopy.

### RESULTS

In light microscopy several sites with doubtless compression artifacts were found in human biopsies (Figs 1-3). At the very site of compression the biopsy appeared lighter than in the surrounding zone that was composed of nerve fibres in which the axon was totally or



brane by treatment with SDS Free carbohydrate which is not bound to SDS or to an SDS-protein complex would not enter the gel during electrophoresis as it would not have a sufficiently high charge to mass ratio and so it would remain undetected even though it might be present in the sample

## ACKNOWLEDGEMENTS

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## ZUSAMMENFASSUNG

Die Proteine der normalen Membrana tectoria der Maus wurden durch SDS Polyacrylamid-Gel-Elektrophorese analysiert Drei wichtige Proteinblöcke mit Molekulargewichten von 145 000 155 000 und 165 000 und eine Anzahl kleinerer Teile wurden gefunden Abnorme Membrana tectoria von Mäusen die man mit Thioracil behandelte und von Deafness Jerke und Variet Waddler Mutanten wurden auch in dieser Weise geprüft aber es gab keinen Beweis die Hypothese zu bevorzugen, daß eine abnorme Proteinbeschaffenheit die Verzerrung der Membranen hätte verursachen können. Kein PAS-positives Material ließ sich in Gelen der normalen Membranen demonstrieren

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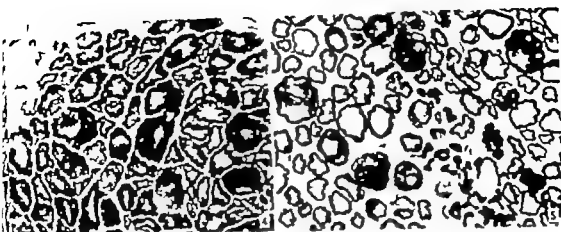


Fig. 4. Peripheral parts of biopsy taken from the vestibular nerve of normal male rat with a biopsy forceps before perfusion fixation. Note thickening of the myelin in many fibres and replacement of the axoplasm by myelin material.  $\times 687$ .

Fig. 5. A biopsy of a rat vestibular nerve taken before perfusion fixation. There are numerous changes that in most

cases should be considered artifacts. At several places there is evidence of rupture of the myelin layer, widening of the nerve fibres and myelin material replacing parts of the axoplasm inside the myelin coat. Artifactual tearing of capillaries has also occurred and erythrocytes are seen between the nerve fibres.  $\times 713$ .

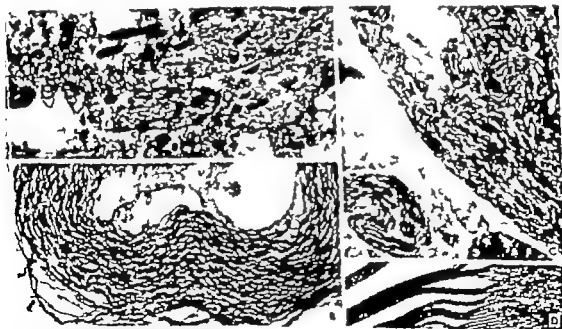


Fig. 6. (A) Decoupled axons in an area which has been punched with biopsy forceps. Around the axons there is granular debris left by the myelin coats and the Schwann cells surrounding the fibres. Biopsy taken before perfusion fixation from the 8th nerve of rat.  $\times 9600$ . (B) Greatly thickened myelin coat of nerve fibre in an area of compression in 8th nerve biopsy from laboratory rat. There are large axonal vacuoles between the myelin coat and the axon.  $\times 7400$ . (C) Human 8th nerve biopsy. Great-

ly thickened myelin layer with convoluted groups of lamellae which also show separation in places. The axon is compressed at the periphery of the fibre. The greatly thickened myelin layer with condensed myelin lamellae were more common in samples showing evidence of compression than in samples which did not.  $\times 8400$ . (D) Separation of myelin lamellae at the minor dense lines.  $\times 8000$ .

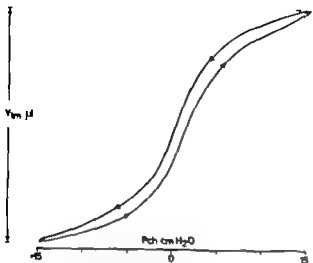


Fig. 1 Recording of the volume/pressure curve of the tympanic membrane system. The recording shows how the volume/pressure curve follows different tracks when the pressure decreases and increases—hysteresis.

using a pressure chamber and the method worked out by Ingelstedt et al (1967) and Elner et al (1971a). The main principle of the method is to record the volume deviation of the tympanic membrane in relation to its neutral position at changes of ambient as well as of middle ear pressure. The method also makes it possible to study the elastic properties of the tympanic membrane system recorded as a volume/pressure relationship. This quotient is defined as the volume deviation of the eardrum  $V_{tm}$  in relation to its neutral position and as a function of pressure differences across the eardrum  $V_{tm} = f(P_{tm})$ . The method has been described in detail by Elner et al (1971c).

In the present study the volume/pressure relationship was recorded within a pressure range of  $\pm 15$  cmH<sub>2</sub>O. The Eustachian tubes were closed during the recordings and the chamber pressure was kept constant at +15 and -15 cmH<sub>2</sub>O for 3 sec (Fig. 1). The total volume displacement of the tympanic membrane  $V_{tm}$  was calculated between these static pressure points. This pressure range 30 cmH<sub>2</sub>O was the same as that used by Elner et al (1971c).

The material was divided into four different

tubal function groups according to Elner et al (1971b).

Group I equilibrates pressure difference across the eardrum completely.

Group II equilibrates pressure difference with small residual pressure in the middle ear.

Group III equilibrates a relative overpressure but not an underpressure.

Group IV is incapable of equilibrating over and underpressure by deglutitions.

Group I was divided into subgroups

Ia patulous tube

Ib equilibrates over and underpressure of 10 cmH<sub>2</sub>O in the middle ear by 1–3 deglutitions.

Ic equilibrates over and underpressure of 10 cmH<sub>2</sub>O in the middle ear by 4–10 deglutitions.

## MATERIAL

The material consisted of 24 experienced scuba sport divers (46 ears) 26–34 years old (mean 29) with normal ear findings and without any signs of catarrhal infection on the day of the experiments. They had been diving for about 5 years with a mean of 350 logged dives. Twenty divers had a normal hearing within 0–20 dB (related to ISO standard 1964). Four divers had a sensorineural hearing loss. The hearing loss was known since early youth in two of the divers. The results were compared with the results obtained from 98 subjects (98

Table 1 The distribution in different tubal function groups

| Tubal function groups | Divers      |           | Non-divers  |           |
|-----------------------|-------------|-----------|-------------|-----------|
|                       | No. of ears | $\bar{x}$ | No. of ears | $\bar{x}$ |
| Ib                    | 18          | 40        | 13          | 34        |
| Ic                    | 12          | 37        | 37          | 38        |
| II                    | 11          | 4         | 21          | 1         |
| III/IV                | 4           | 9         | 7           | 7         |
| Total                 | 45          | 100       | 98          | 100       |

icular degeneration of the myelin found in spinal cord trauma, corresponds to the separation of myelin lamellae in the thickened myelin coats in our study and appears to be initiated by mechanical damage. The full development of this change and the development of vacuoles inside myelin appears to be due to active changes in fluid compartments of the nerve fibres. We did not observe such a change probably because the samples were fixed immediately after biopsy.

## ZUSAMMENFASSUNG

Das Vorkommen von triebfaßartigen Veränderungen an Biopsien vom 8. Hirnnerv des Menschen wurde untersucht und entsprechende Veränderungen wurden in Biopsien von Ratten durch mechanische Behandlung vor der Fixation verursacht. Die Färbung der Nervenfaser war schwächer an der Stelle der Kompression als in der Umgebung. In elektronenmikroskopischen Untersuchungen beobachtete man hier fragmentierte Axone und vakuolisiertes Debris. Ferner in der Umgebung beobachtete man verdickte Markscheiden und in vielen Fällen konnte man das Axon nicht mehr sehen. Die Myelinlamellen waren teilweise separiert und oft in runde Porenböden reorganisiert. Veränderungen in Diametern der Nerven und Rupture der Markscheiden wurden auch nach der mechanischen Behandlung vor der chemischen Fixation beobachtet.

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Das Verhältnis zwischen Volumen und Druck im System des Trommelfells wurde bei 4 Sporttauchern untersucht. Die Befunde wurden mit Befunden an 98 Nichttauchern verglichen. Unter den Personen mit guter Tubenfunktion hatten die Taucher im Vergleich zu den Nichttauchern eine vermehrte Beweglichkeit des Trommelfellsystems. Taucher mit reduzierter Tubenfunktion unterschieden sich nicht von Nichttauchern mit reduzierter Tubenfunktion. Faktoren, die das Verhältnis zwischen Volumen und Druck im System des Trommelfells beeinflussen, werden diskutiert.

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Fig. 3. Detail of Fig. 3. The organ of Corti and the ganglion cells are absent.

reliable. This check was made on both sides. The destruction was considered complete when no microphonic or action potentials higher than  $5 \mu\text{V}$  could be measured. A fenestrum was made in the lateral canal on one side near the ampulla. An electrode was placed on the edge of the fenestrum and when microphonic and APs were detectable an equal response curve for  $10 \mu\text{V}$  was recorded for the microphonic.

In the week after the fenestration the nystagmogram and the Tullio reaction were checked and the animal was again conditioned to sound. Then, after about a week, we tried to obtain a behavioural response to sound by means of a shiver audiogram.

We observed the animals for about 3 months

after fenestration. At the end of the program the animals were sacrificed and prepared for microscopic examination.

#### Experiments with pigeons

From seven 1 year-old pigeons of about 600 g the cochlea was removed. In contrast to the guinea pig this can easily be done in a pigeon without destroying the vestibular organ (Bleeker & de Vries 1949; van Eyck, 1955). The same anaesthesia with oxygen and fluothane was used. The skull was opened in the anterior inferior part of the cross formed by the posterior and lateral canal (Ewald, 1892). After removal of the external plate of the skull the recessus scalae tympani, the columella, the round window and the labyrinthine canals

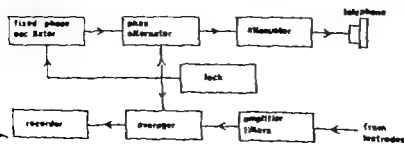


Fig. 4. Apparatus for measurement of microphonic and action potentials from pigeons.

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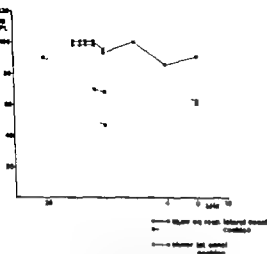


Fig. 7. Gomes pig 15. Equal response curves of the microphonics and shiver thresholds obtained from the intact cochlea and from the lateral canal after bilateral destruction of the cochlea and fenestration of the lateral canal.

cedure took much time because three operations on each animal were necessary. This created many opportunities for failure. Some animals died during the procedure, others developed otitis. Sometimes the laser radiation was too intensive, causing damage to the labyrinthine structures. In other cases the radiation was too weak, causing incomplete destruction of the cochlea and a rest effect of cochlear microphonic and action potentials. Of the 20 animals, only two showed a significant and clear behavioural response together with the electrical responses after fenestration, a response not obtainable before fenestration and after destruction of both cochleae.

Figs 11 and 7 show the results in one of the animals (no. 15). The fact that the shiver audiogram showed behavioural response for frequencies as high as 8 kHz, whereas the fact that microphonics above 1 kHz were not obtainable can possibly be explained by supposing that the animal reacted at the onset of the acoustic stimulus. The second animal (no. 18) showed nearly identical curves. Fig. 8 gives the input-output curve for the compound action potential for one of the guinea pigs measured before destruction of the cochlea (the

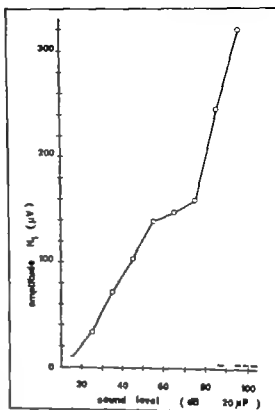


Fig. 8. Amplitude of compound action potential (N) before destruction of the cochlea. The amplitude was below the dotted line (5  $\mu$ V level) after destruction of the cochlea.

other animals gave similar results). In spite of the simplicity of the apparatus used to measure these action potentials, a large degree of malfunction of the cochlea after laser radiation could be proven with it.

#### Pigeon experiments

In the second program, electrophysiological results were obtained from 7 pigeons. The results of the microphonic measurements are given in Figs 9 and 10. The latter figure shows clearly that microphonics have almost disappeared after extirpation of the cochlea. After fenestration, much larger microphonic-like signals could again be detected. Before extirpation of the cochlea, action potentials could be obtained with high as well as with lower stimulus frequencies (Fig. 11). After ex-



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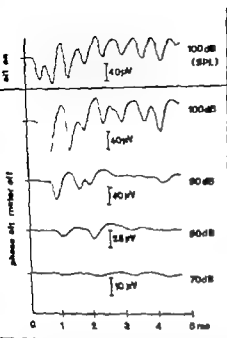


Fig. 12. Action potentials from a pigeon after cochlear extirpation and fenestration. The upper part of the figure is obtained with 1000 Hz tone burst (4 ms duration) delivered to the telephone with alternating phase. For the lower part of the figure the phase alternator was switched off.

levels. Before cochlea extirpation the action potential latency ranges from 0.9 to 2 ms.

### CONCLUSION

This work is a preliminary report. With these few results we can only indicate that in guinea pigs and pigeons with total cochlear loss the labyrinth might perhaps give some meaningful sonic information after fenestration of the lateral canal.

On account of the simplicity of the operation, fenestration of the lateral canal in patients with total cochlear loss on both sides can be considered as an alternative to monopolar cochlear implants. The middle ear can be left intact. There is no need to create a cavity as in the original fenestration operation for otosclerosis. Caloric vestibular disturbances are therefore of minor importance. Closure of the round window to ameliorate the efficiency

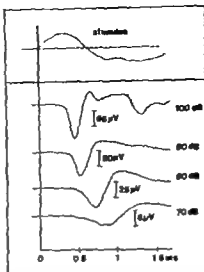


Fig. 13. Action potentials from a pigeon after cochlear extirpation and fenestration. The sound pressure of the stimulus is given in the upper part of the figure (same time scale as lower part). Stimulus level is given as peak equivalent sound pressure level.

of the fenestration might be considered. We can only guess about the meaning of this vestibular signal obtained by sound stimuli. It can hardly be expected that frequency discrimination is possible—apart perhaps from very low frequencies. But the quality of the information might be equal to that obtained by monopolar cochlear implants.

### ZUSAMMENFASSUNG

In einem Bericht von Untersuchungen an Meerschweinchen und Tauben werden die elektrophysiologischen Resultate und das Reflexverhalten von akustischer Reizung des Labyrinthes geschildert. Beim Meerschweinchen waren beiderseits die Schenkel ausgeklappt mittels Laserstrahlung und der laterale Kanal fenestriert. Die Schreck der Tauben wurde vor der Fenestration cateniert. Auf Grund der Resultate wird darauf hingewiesen daß möglicherweise bei Patienten mit totalem Ausfall des Gehörs hindernisse mittels einer Fenestration des lateralen Kanals ähnliche Resultate erreicht werden können als mit monopolarer „cochlear implants“.

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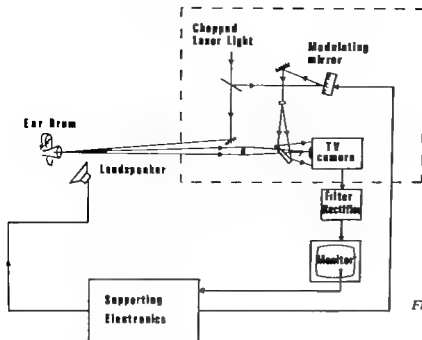


Fig 1 ESPI design

can be simply said to represent the object's position relative to a reference point measured in terms of the wavelength of the laserlight. If the object changes its position during the hologram exposure, the resulting change in phase relationship is recorded by the holographic process. When afterwards the reconstructed object is seen through the hologram, the object is covered with a pattern of dark and bright fringes. This fringe pattern can be used like contours on an ordinary map to determine the object's movement. When holography is used for such measuring purposes, it is called holographic interferometry. Holographic interferometry is a very sensitive measuring technique as the wavelength of light is used as the unit.

Tonndorf & Khanna (1972) used holographic vibration analysis in investigating the movements of the drum in the cat. Gundersen & Høgmøen (1976) used the same method in vibration measurements on human temporal bone preparations.

However, the holographic process is slow and complicated because it is based upon exposure and development of high resolution filmplates.

If the film registration process is replaced by TV camera recording with subsequent elec-

tronic filtering and display on a TV monitor, we gain immensely in speed and ease of handling. Exposure time is short (1/25 sec) and a new hologram is also created every 1/25 sec. The TV holography system, which is usually called electronic speckle pattern interferometry or ESPI, is very insensitive towards objects' instability and external vibrations. It is therefore well suited for work on unstable objects like ear preparations (Høgmøen & Løkberg 1977).

The background for this investigation is our work on increasing the application range of ESPI for vibration analysis, mainly based on reference wave modulation techniques. Recently we have applied ESPI to measure the vibratory motion of the eardrum in living man.

## METHOD

Fig 1 gives a schematic presentation of our ESPI design. ESPI is a holographic method which uses the target of a TV camera as the registration medium for the interference patterns. The hologram reconstruction is performed electronically by filtering and rectifying the video signal from the camera, and the resulting interferograms are presented in real-

ANALYSIS OF THE MECHANICAL IMPEDANCE  
OF BONE ANCHORED HEARING AIDSA Tjellström E Håkansson\* J Lindström P J Brånemark,  
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From ENT-Department Sahlgren Hospital, University of Göteborg Göteborg \*Laboratory of Medical Electronics  
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**Abstract** Some patients who need hearing aids are unable to use an apparatus which transmits the sound via the external ear canal and have to use a bone conduction hearing aid. The bone vibration transducer of this aid is applied to the skin over the mastoid process and the sound is transmitted via the soft tissue and bone to the cochlea. The pressure needed to apply the transducer often gives the patient discomfort and the damping effect of the soft tissue gives poor quality of the sound transmitted. Advances in the ability to permanently implant foreign material in the body and perform permanent skin penetration has made it possible to develop bone-anchored hearing aid. Fourteen patients have been equipped with such hearing aids. They are able to give these patients the best hearing aid, new transducers has to be constructed to match the new situation. The impedance of the bone-anchored titanium screw/staple has been studied and the resistance and reactance of the mechanical impedance have been measured. The influence of damping soft tissue layer over the bone has been analyzed. The difference between the impedance of the skull and the impedance of the soft tissue + skull was in the order of 10 to 25 dB depending on the frequency.

For various reasons some patients who need hearing aids cannot use an apparatus in which the sound is transmitted via a plastic ear mould in the external auditory canal but require a bone conduction hearing aid instead. This means that the sound is transmitted to a bone conduction receiver applied to the skin over the mastoid process by means of a steel spring over the head via soft tissue and bone into the cochlea. One reason why the patients must use such a hearing aid can be a chronic ear condition and that the ear starts to discharge as soon as the auditory canal is blocked by an ear mould. Other reasons are extreme transmissi-

sion impairments such as gross malformation of the ear canal and the middle ear and also extensive otosclerosis.

Patients with bone conduction hearing aids often complain about the pressure with which the hearing aid must be applied. This pressure is so great that it causes pain and one often sees reddening and irritated skin areas which indicate where the hearing aid has compressed the skin. The position of the apparatus must often be adjusted so that the patient can get the best hearing. This may be due to the roughness and convex nature of the bone surface under the skin which also hinders an exact and stable position of the hearing aid. Many patients complain about the poor sound quality which characterizes this type of hearing aid. Further the steel spring over the head for holding the hearing aid in position can give esthetic problems for thin-haired men as well.

This paper is the first in a series of presentations concerning an inter-disciplinary development program on bone-anchored hearing aids. The work is done in close cooperation between the Department of Otolaryngology University of Göteborg (Head Prof. Ole Hallén), The Institute for Applied Biotechnology Mölndal (Head Prof. P-J Brånemark), The Department of Audiology University of Göteborg (Head Prof. G. Lidén) and the Laboratory of Medical Electronics, Department of Applied Electronics, Chalmers University of Technology Göteborg (Head Prof. R. Magnusson). This study was supported by grant from Sanktens Tysta Skolan, Stockholm, The Swedish Medical Research Council, project No B 79-17X-05436-01 and The Göteborg Medical Society Göteborg.

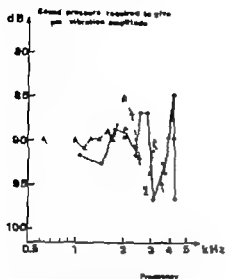


Fig. 3 Frequency response of the malleus manubrium tip of male subjects. (Sound pressure level given in dB re  $2 \cdot 10^{-8}$  N/m)

lated linearly as indicated which eased the alignment procedure considerably. In addition the subject could watch the tympanic membrane interferograms on a separate TV monitor which enhanced advantageous co-operation.

The ear tube was painted dull black on the inside but no precautions such as spraying with bronze powder were taken to improve the optical properties of the membrane itself. The optical properties of the object are difficult, with variations in reflectivity across the membrane and with a large component of internally reflected light because it is semi-transparent. This would ordinarily result in very low contrast in the interference patterns. But by modulating the reference wave with an amplitude that corresponded to a zero point of the time average characteristic fringe function (the 1st and 2nd zero point were used) this problem was solved since the unwanted light normally coincided with zero intensity. Light reflected from parts of the membrane that vibrated with the same amplitude and phase as the reference mirror however would coincide with maximum intensity.

The eardrum was stimulated by the free sound field from a loudspeaker. The sound pressure level (SPL) was monitored by a

microphone positioned close to the ear. SPL values cited in this paper must be taken with care as they varied quite considerably with the position of the microphone. In future work the SPL should be measured near the drum as possible to give reproducible results.

During the experiments extensive use was made of a videotape recorder for subsequence analysis of and measurements on the recorded interferograms. However the real-time nature of the ESPI was absolutely necessary for all the adjustments of different parameters such as reference wave modulation, object vibration and ESPI parameters which had to be performed during the experiments.

Photoelectric measurements from the recordings were made to obtain absolute amplitude values at low sound pressures such as 70–80 dB with amplitudes in the range of 1 and below. Phase variations across the eardrum were also measured.

## RESULTS

The preliminary experiments have been undertaken to demonstrate that ESPI with a continuous laser can be used to measure drum vibration on living objects. The experiments have also been performed under improvised conditions which do not involve a large series of measurements.

Fig. 3 shows the frequency response of the malleus manubrium tip of the left ear of two male subjects. It gives the SPL necessary to obtain the first dark fringe (corresponds to  $0.1 \mu\text{m}$  amplitude) as a function of frequency. The shape of the curve is similar to the curves Gundersen (1971) observed using an electromagnetic pick-up.

Both subjects showed a point of resonance in the region of 2 kHz. In temporal bone preparations the point of resonance was between 800 and 1200 Hz. The shifting of the point of resonance to higher frequencies in living man may be due to contractions of the muscles of the middle ear.



Fig. 2. Input impedance to the head ( $Z$ ) where  $Z_{sk}$  corresponds to the impedance of skin and subcutaneous tissue and  $Z_s$  corresponds to the impedance of the skull.

months and no fixture has loosened from the bone (February 1979). Depending on the immature technical design, a couple of the patients have complained about minor inconvenience from the fixtures. The hearing aids used by these patients have been of the same manufacture and the same type as their usual hearing aids.

The bone-anchored hearing aid operates with a changed input impedance looking into the head. The input impedance together with a given hearing aid with its associated electronics determines the input power to the head. The variation of the power with frequency is different for bone-anchored transmission and conventional skin and bone transmission. The patients used the same manufacture and type of hearing aids as before and most of them feel that the sound has a better quality for bone-anchored transmission than for conventional bone transmission. Optimization of the hearing aid should lead to a further increase in the sound quality. Such optimization requires a closer study of the impedance in the system. Psychocoustic analysis must be carried out in order first to further develop the hearing aid and later to match the associated electronics.

The purpose of the study present here is to describe and investigate the mechanical system when an osseointegrated entrance to the skull is used. A comparison with the ISO/IEC standard for mechanical impedance in skin-head will also be carried out (Flottorp/Solberg, 1976).

#### Models—electrical analogs

Mechanical systems, of which sound transmission is one, are complicated and can best be

described by models. These can be mechanical and can also be translated to electrical analogs. The mass corresponds to an inductance ( $L$ ), the elasticity to capacitance ( $C$ ) and damping to resistance ( $R$ ). The head with soft tissue and bone supported by soft tissue of the neck and neck bones can be considered a mechanical system. To study this system two general models have been proposed. In one of the models shown in Fig. 2, the impedance of the skin and subcutaneous tissue is described by  $Z_{sk}$ . In the other model shown in Fig. 3 the impedance of the snap fastener is described by  $Z_{sf}$  and  $Z$  describes the impedance of the bone of the skull. The impedance of the osseointegrated titanium fixture is negligible because its mass is very small and the physical properties of titanium correspond well with those of bone. The construction of the snap fastener is shown schematically in Fig. 1. These two models describe the input impedance into the head in the frequency range 250 to 8000 Hz, with and without skin penetration respectively. If the mechanical snap fastener is stiffer than the skin including the subcutaneous tissue over the mastoid process the snap fastener system  $Z$  is better than the system with subcutaneous tissue  $Z_{sk}$ .

#### MEASUREMENT APPARATUS AND METHOD

The measurements were made with Bruel & Kjaer's impedance measurement apparatus consisting mainly of an impedance head and a vibrator. A block diagram of the measurement apparatus is shown in Fig. 4. The impedance of the mass in the driving platform in the impedance head has been taken into account.



Fig. 3. Input impedance to the head ( $Z$ ) where  $Z_{sf}$  corresponds to the impedance of the snap fastener and  $Z_s$  corresponds to the impedance of the skull.

rungszeit jedes Fernsehbildes erreicht. Die Amplituden und Phaseverteilung des Trommelfells wurden mittels der Phasemodellierungstechniken erfaßt, die auch die Schwingungen im Zeitlupentempo darstellen konnten. Bei photoelektrischer Aufdeckung der Intensität des Fernsehbildes konnten Amplituden bis zu  $\mu\text{m}$  gemessen werden. Vorläufiger Erfolg wird präsentiert.

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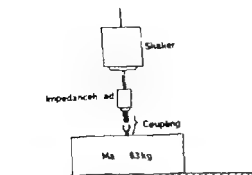


Fig 6 Measurement apparatus for impedance measurements on snap fastener only

## RESULTS

The results of the magnitude (in mechanical ohms) and phase (in degrees) measurements are presented in Figs 7 and 8. The curves show an average of the measurement results in eight patients. A scattering in the magnitude measurements of about  $\pm 30\%$  is noted for frequencies below 1250 Hz and over 5000 Hz. Between these frequencies the scattering is less than  $\pm 12\%$ . The bump at 1250 Hz is relatively flat in some cases. The curves obtained for the various patients all had the same

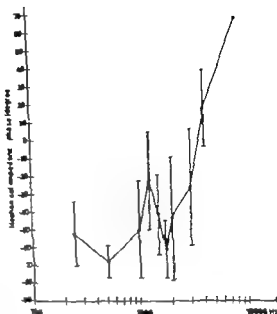


Fig 8 Phase of the mechanical impedance (average). The scatter of the 8 patients is shown

characteristics. From the magnitude and phase curves the resistive and reactive impedance have been calculated and are shown in Fig. 9. The scattering of phase is larger than that of magnitude which is caused by greater indi-

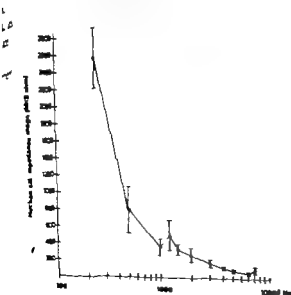


Fig 7 Magnitude of the mechanical impedance (average). The scatter of the 8 patients is shown

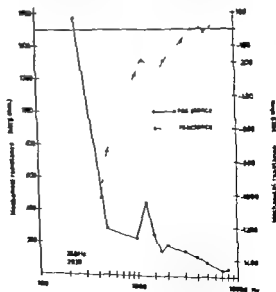


Fig 9 Resistance and reactance of the mechanical impedance



rungszeit jedes Fernschbildes erreicht. Die Amplituden und Phaseverteilung des Trommelfells wurden mittels der Phasemodellierungstechniken erfaßt, die auch die Schwingungen im Zeitlupe tempo darstellen konnten. Bei photoelektrischer Aufdeckung der Intensität des Fernschbildes konnten Amplituden bis zu 2 nm gemessen werden. Vorläufiger Erfolg wird präsentiert.

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## DEFECTS OF THE OTOCONIAL MEMBRANES IN NORMAL GUINEA PIGS

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(Received March 23, 1979)

**Abstract** Otoconial membranes from a large number of normal albino and pigmented guinea pigs were examined by light microscopy. In more than 20% of the animals, microdissection revealed that the crystalline layer of the otoconial membrane was incomplete, leaving large areas of the underlying gelatinous layer uncovered by crystals. These defects were bilateral, more or less symmetrical, and invariably more extensive on the saccule than in the utricle. In two animals, large single conglomerates of crystals were found on both the saccular and utricular maculae. None of the animals displayed abnormal behavior or posture and no obvious cause for the otoconial defects was found. The presence of hereditary congenital deafness is postulated. The high incidence of otoconial defects in guinea pigs makes them poorly suited for most investigations of experimentally induced pathology. Therefore, previous reports of otoconial pathology in guinea pigs should be viewed in the light of these findings.

The purpose of this paper is to report what appears to represent a marked reduction of saccular and utricular otoconia in a large number of untreated guinea pigs which were used as normal material in our laboratory. This unexpected finding was made during dissection of the vestibular system in 168 guinea pigs for a study of the incorporation of  $^{45}\text{Ca}$  in the otoconial membranes.

The normal appearance of the otoconial membrane in the guinea pig has been described by several investigators who have used the technique of microdissection, which allows a direct inspection of the otoconia (Engström et al. 1966; Hawkins & Johansson 1976). The most detailed description is given by Lindeman (1969) and the reader is referred to his monograph concerning the normal morphology of the vestibular apparatus. The otoconial

membrane in man has been described in adults (de Burlet & Hoffman 1929; Johansson & Hawkins 1967) in infants (Wright & Hubbard 1978) and in fetuses (Wright, Hubbard & Clark, 1979a). Scanning electron microscopy has given more precise information about the shape and structure of the otoconia in man (Ross et al. 1976; Wright & Hubbard 1978) as well as in rodents (Kellerhals et al. 1970; Lim, 1973a, b; Ross & Peacor 1975).

In most previous descriptions and illustrations of the normal macular organs, the otoconial layer covers the entire surface of the neuro-epithelium. The layer of otoconia is continuous, appears snow white and is strikingly displayed. The surface is uneven and the otoconia form a characteristic ridge or crest, the so-called snowdrift, as it is referred to by Engström et al. (1966). The curvatures of the snowdrift lines in man and guinea pigs are similar in shape. In the saccule the line is gently curved while it is more horseshoe or "U" shaped in the utricle (Fig. 1). The snowdrift covers approximately Werner's (1940) *striola* ('little stripe'), i.e. the area of the neuro-epithelium containing the greatest concentration of Type I hair cells.

Little is known about the possible metabolism of the otoconia. Uptake of  $^{45}\text{Ca}$  by the otoconial membranes in gerbils (Preston et al. 1975) and in guinea pigs has been demonstrated. The incorporation of  $^{45}\text{Ca}$  can be altered by administration of ototoxic drugs (McChigian et al. 1979). These findings suggest growth and neogenesis of otoconia. Lim

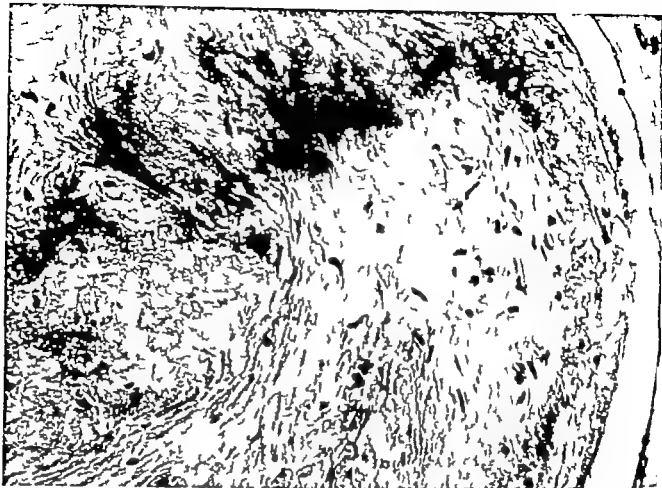


Fig 1 Tympanosclerosis with increase of collagenous fibres hyalinization and calcification (Haematoxylin-eosin  $\times 135$ )

Rennie (1963) claims that there is less wrinkling and detachment of de-paraffinized sections

#### *Electron microscopical preparation*

Identical samples were placed in phosphate buffered 3% glutaraldehyde (pH=7.2) for 3 h at room temperature and post fixed in 1% buffered  $\text{OsO}_4$  for 2 h at 4°C. After post fixation the tissues were dehydrated through ethanols and propylene oxide and embedded in Epon 812. Sections were stained with uranyl acetate and examined in a Zeiss EM 9.

## RESULTS

### *Histology*

The histological observations with the haematoxylin-eosin stain resembled the descrip-

tion of other investigators (Beck & Ebert 1964) increase of collagenous fibres hyalin degeneration and calcium deposits (Fig. 1).

The connective tissue was poor in cells. The dominant cell type was fibroblasts. The vascularization was sparse. There was no sign of granulation tissue. The overlying epithelium in some locations was intact; in some locations there was a defect.

### *Histochemistry*

Material which reacted with aldehyde fuchsin but not Azan, orcein or periodic acid-Schiff sequence was seen in previously oxidized sections from all tympanosclerotic plaques (Fig. 2a, b). According to their histochemical definition (Fullmer et al. 1974) these fibres are oxytalan fibres.

In tympanosclerosis the fibre distribution

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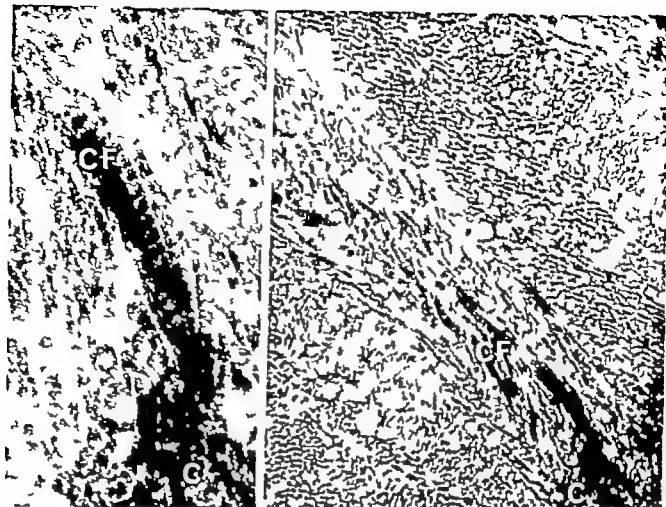


Fig 3a Abnormal large collagenous fibrils with atypical cross-linkages. CF=collagenous fibrils, C=calciocalbon

( $\times 1120$ ) 1 set Higher magnification of the indicated area in fig 3a ( ) ( $\times 63360$ )

was not uniform but showed rather focal aggregations. There was multiple branching (Fig 2c) and related necrosis. Oxytalan fibres were not seen in vicinity to blood vessels and were never observed in intact tissue.

#### Electron microscopy

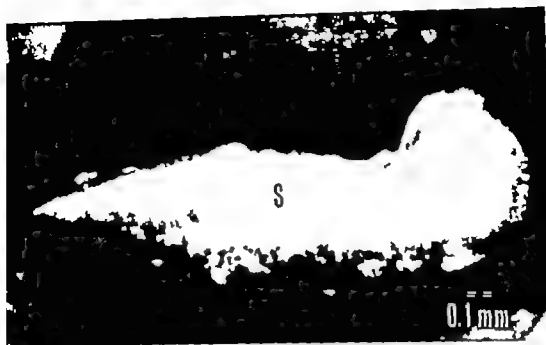
**Extracellular space** The numbers of collagenous fibres were increased. In most cases their elements ran in various directions, loosely or densely packed together.

In addition, foci of degraded collagen fibres appeared. These elements were characterized by an increasing loss of the typical fibril periodicity and by fibril swelling. An amorphous electron-dense material was often seen in the matrix. It tended to be concentrated either about areas where collagen fibrils appeared to

be swelling and losing their characteristic banding pattern or in areas where collagen fibrils were fragmented and appeared to be undergoing dissolution (Fig 3a, b).

In close vicinity to the borders of the collagen fibrillar bundles and the zones of amorphous material, aggregations of thin microfibrils measuring 100–150 Å in diameter were observed. When viewed in cross section they were seen to be assembled parallel with the fibre axis (Fig 3b, 4).

Within the extracellular space, membrane-bound corpuscles were seen containing amorphous electron-dense material and perimembranous haloes. They could be located either in otherwise empty niches or in between the collagen fibril bundles. No relation was found to the microfibrillar elements. Vesicles ap-



come easily dislodged post mortem (Wright & Hubbard 1978) and numerous single otoconia tend to fall off the membranes during the process of dissection. These types of artifacts are usually easily identified, in contrast to those which may be caused by the various agents used during the processing of labyrinthine tissue. Werner (1934) first reported that fixation at low temperatures ( $10^{\circ}\text{C}$ ) dissolves the saccular otoconia, while the utricular otoconia remain intact. His observation is still confirmed by other investigators. It was initially felt that the now established technique of microdissection which involves staining of the inner ear with neutral osmium tetroxide solution left the otoconia undisturbed. Later several investigators expressed concern about the possibility that the subsequent dehydration and storage in alcohol may alter the otoconia.  $\text{\AA}$ im (1973a) actually published a photomicrograph of 'collapsed' otoconia which had been stored for one week in alcohol. The question of the possible occurrence of artifacts due to the processing of temporal bones for micro-

dissection remains unanswered. Our feeling is that as long as all solutions including the alcohol are kept neutral the process does not significantly alter the otoconia. It should be kept in mind however that alcohol becomes oxidized and acidic with time. The small amount of buffer remaining from the fixation solution is usually sufficient to maintain a neutral pH for some time. We believe however that long term storage in alcohol should be avoided if a study of the otoconia is intended. At present we prefer to examine the otoconial membranes at least in a part of our material within a day or two after fixation.

## METHODS

The specimens were stained with Millonig or Zetterquist's osmium tetroxide solution (pH 7.2-7.4). Some specimens were stained for 1 to 24 hours while others were stained for 1 to 4 hours or less. After staining a partial dehydration was performed with ascending

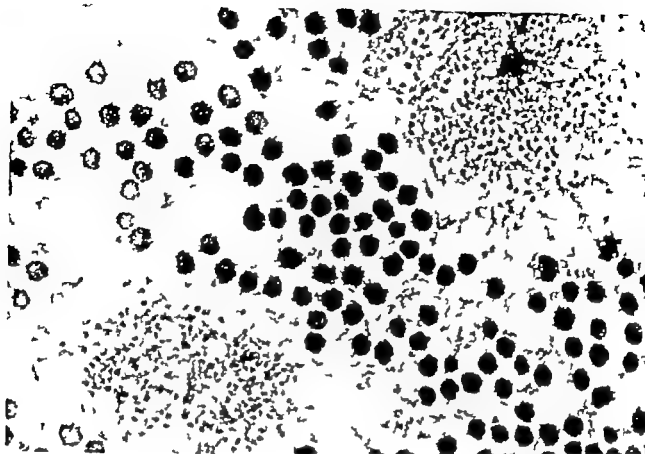


Fig. 4 Cross-section of oxytalan microfibrils in between collagenous fibrils ( $\times 76\,440$ )

tissue. Regarding the collagenous fibres we found both disintegration, increased fibre accumulation and in addition amorphous electron-dense material, probably degraded collagen.

The microfibrillar aggregations seem to be identical with the oxytalan fibres (for ref. see Fullmer et al. 1974). Evidence for the identification of these fibres is based on their histochemical properties and on the ultrastructure of their microfibrils (thickness approx. 100–150 Å).

Because of their histochemical and enzymatic characteristics, oxytalan fibres are accepted as structural glycoproteins which are strongly cross-linked (Fullmer et al. 1974). The physiological significance of these fibres seems to be an intermediate element of fibrogenesis which can hardly ever be detected in normal specimen. Therefore the focal accumulation of oxytalan fibres in the tympano-

sclerotic tissue must be assumed to be a sign of a pathologic ground substance with disturbance in the cross-linkage of the microfibrils. According to Winkle et al. (1973) the collagen fibre increase is similar to the oxytalan fibre production, primarily the result of a disturbed extracellular fibrogenesis and less the result of an augmented cellular activity.

The presence of cellular debris and matrix vesicles is also characteristic of tympanic sclerosis. The lysosomal nature of the matrix vesicles has been demonstrated in other tissues (Riede & Staubesand 1977). As with other tissues, the matrix vesicles in tympanic sclerosis may be sequestered from the cell into the ground substance (Rabinovitch & Anderson 1976) or result from cell degeneration (Spycher et al. 1969; Tyberg 1974). They may be classified as Type I or Type II matrix vesicles, the latter showing a strong affinity to calcification.

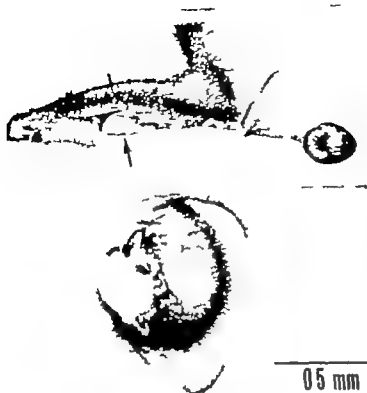


Fig. 5 The gelatinous layer of the otoconial membrane: right ear from the macula sacculi (top) and macula strioli (bottom). No normal-appearing otoconia are present, leaving the translucent membranes exposed with the reinforced, darker area above the striola clearly seen in both specimens. A single large 'otolith' consisting of conglomerate of calcified otoconia is present on each membrane. In the saccule the mass is attached to the accessory membrane (top right). Arrow indicates the original site of the otoconial mass. O+O.

this reinforced line was actually raised from the neuro-epithelium so that a shallow groove was formed on the underside of the membrane along the striola. Cross sections were not made of the membranes: therefore the precise relationship between the groove on the superior and inferior surface was not determined.

In guinea pigs the dorsal extension of the macula sacculi tended to be less pronounced than in man. The gelatinous layer of the membrane had a loose flat appendix of irregular shape extending from the inferior margin just opposite the dorsal extension or from the corner where the anterior and inferior margins meet (Fig. 5). The length of this structure was highly variable. We are not sure whether it is present in all animals. Usually the appendix was shorter than the distance from the tip of the dorsal extension to the inferior margin but in some animals it was longer and could extend

posteriorly halfway along the macula. When the extension was long it rested along the inferior margin of the macula against the membranous wall to which it was sometimes slightly adherent. Lim et al (1974) and Lim & Erway (1974) have referred to this structure as the accessory membrane. It is of interest to note that the accessory membrane was usually covered by a thin layer of otoconia, although it was not in contact with the neuro-epithelium.

#### *Abnormal otoconial membranes*

Differentiating between normal and defective specimens was simple and unequivocal. There were no borderline cases. In the animals with an incomplete set of otoconia, the defects were always obvious and more or less symmetrical. None of our animals had unilateral otoconial defects. Furthermore the abnormal-



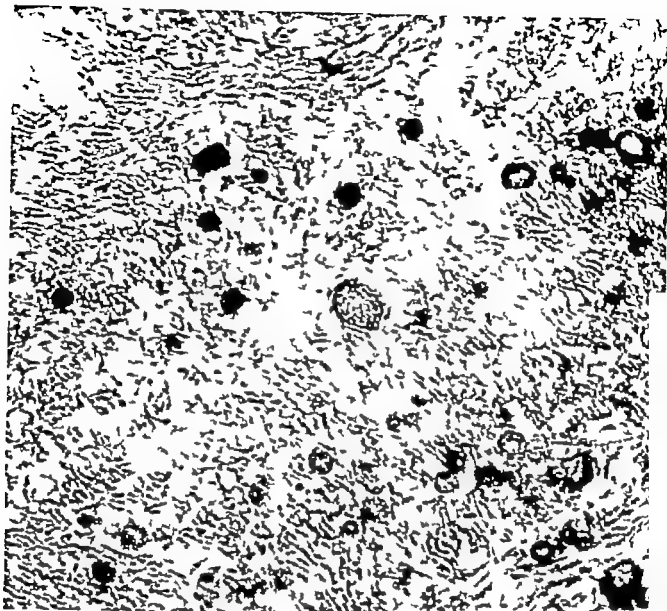


Fig 5b Multiple calcified matrix vesicles Type II ( $\times 5970$ )

in tympanosclerosis there seems to be such an alteration of the fibroblasts that lysosomes are released in increasing numbers into the extracellular space. Such lysosomes are beyond cytoplasmic control and lead to a focal lysosomal activity. In consequence there is a surrounding cellular necrosis and a release of matrix lysosomes leading to a self perpetuation of the inflammatory process. Similar behaviour is described for the myocytic mediolysis and the chondrotic chondrolysis (Riede & Staubesand 1977). Underlining this hypothesis for the causal pathogenesis of tympanosclerosis is the coincidence of  $D_2$  hypervita-

minosis and tympanosclerosis (Chang *et al* 1977). Under these experimental conditions the increased lysosomal activity is the basis of the calciphylaxis (Riede & Staubesand 1977).

As a working hypothesis the effect of such lysosomes beyond cellular control is twofold.

1. There are changes in the fibrillar components: increased collagen formation, degradation of collagen and augmentation of oxytalan fibres. These alterations are predominantly attributed to the presence of Type I vesicles. Type I vesicles are lysosomes containing acid phosphatases. Their enzyme activity leads to degradation of the protein com-



Fig. 6. Surface preparation of the membranous wall of the utricle shows the anterior part of the anastomosing numerous otoconia attached to the wall and centered on the surfaces of single cells. Note to the right

the sharp border between an area covered with otoconia and region free of crystals. Specimen from streptomycin-treated animal. Similar observations were made in untreated animals. OVO. Phase-contrast.

One of these ears had been stored in 70% alcohol for 3 months. The other specimen was dissected the day after sacrifice. It is highly unlikely that the finding represents an artifact. Each giant otoconium was formed by a conglomerate of calcite. The utricular mass had a roundish main body with large crystals protruding from it. The saccular mass was also roundish with an uneven surface formed by innumerable small sharp-pointed crystals.

$^{45}\text{Ca}$  incorporation in the defective otoconial membranes was variable but consistently lower than in normal-appearing membranes. It is not known whether the decreased uptake was due to the smaller total mass of otoconia in these animals or to a decreased calcium metabolism or other factors. The defective specimens were not included in the  $^{45}\text{Ca}$  study (Mechigian et al. 1979).

No systematic examination of the macular neuro-epithelium was done. Surface preparations of the neuro-epithelia were obtained from several abnormal-appearing maculae and examined in phase-contrast microscopy. There was no obvious hair cell loss. No abnormal behavior or posture was observed in these animals. However tests of vestibular reflexes or swimming ability were not performed.

#### *Incidence of abnormality*

In the hope that a type of animal could be found in which there was no occurrence of defective otoconial membranes a series of 3-colored guinea pigs was examined. An attempt was made to correlate incidence of otoconial abnormalities with coat color (black vs. dark

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hic membrane is not known. Perhaps it may help to stabilize the otolithic membrane proper and limit its movements. The otolithic membranes of the macula sacculi and utricle in man do not have accessory membranes (Johnson & Hawkins 1967) nor does that of the sacula utricle in the guinea pig. In gerbils and pigeons we have observed a prominent round buckling of the anterior margin of the otoconial membrane in the utricle. This part of the membrane is not covered by otoconia and is in contact with the membranous wall.

In animals with both normal and abnormal otoconial membranes we found numerous crystals attached to the membranous wall of the utricle. They were particularly numerous in the abnormal utricle and were often seen clustered within the boundaries of single cells. The nature of these cells was not determined; probably they represented dark cells (Lim 1973a). These otoconia were examined only by light microscopy; admittedly it is difficult to evaluate their state so well as is possible in scanning electron microscopy. Because they were highly refractile, mostly small and had the well-defined shape of normal otoconia, we do not believe that these crystals were in a state of decay like the ones pictured by Lim (1973a). On the contrary, it could be argued that the small otoconia were newly formed but their origin and fate is enigmatic.

We did not observe any obvious tendency for the otoconia in the defective utricles to fall into the posterior ampulla and create a condition analogous to cupulolithiasis (Schuknecht & Ruby 1973; Schuknecht 1974). This would in any case be difficult to prove, since otoconia do become dislodged during processing and dissection.

It is hoped that this study will elicit further investigations of the otoconia in normal guinea pig populations. Perhaps the loss of saccular otoconia attributed to fixation in Werner's (1934) study did not represent an artifact—but rather pathology. Lindeman (1969) reported in his monograph on normal vestibular morphology that in some instances otoconial mem-

branes had an unusual appearance and his brief description of these particular specimens suggests that they were similar to the abnormal specimens in our study. Harada & Sugimoto (1977) also noted that the otoconial layer was thinner above the striola region in the utricle of their untreated guinea pigs. They reported that a loss of otoconia occurred along this line after streptomycin administration. Their illustrations of this phenomenon appear similar to those seen in Figs 2-4 in our untreated normal guinea pigs.

The high incidence of otoconial defects in the guinea pig makes this animal poorly suited for investigating experimentally induced otoconial pathology, and previous reports of otoconial abnormalities in guinea pigs should be viewed in the light of the present findings. On the other hand, a careful comparative study of the macular organs in the animals with normal and abnormal otoconial membranes could reveal important information about the formation of otoconia and the structures involved in this process. While the reduction in number of otoconia tended to be more random in the sacculus, it always occurred above the striola in the utricle. The findings might imply a local disturbance of the calcium supply in this region due to some defect in the Type I sensory cells or in their supporting cells.

Assuming that sensory cells under the abnormal otoconial membranes can respond normally to stimulation, the two types of guinea pig could serve as a useful model in neurophysiological experiments concerning the effect of the mass of otoconia on the function of the macular organs.

## ACKNOWLEDGEMENTS

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Fig 1 A phase contrast photograph of human spiral ganglion cells showing small neurons (arrows) and large neurons near the nerve fibers close to the osseous spiral lamina. The small neurons were followed in sequence from the beginning to end in serial sections.

before direct perfusion of 1% phosphate buffered osmium into the inner ear varied from 1-1/2 to 6 hours. The procedure of preparing specimens for electron microscopy was described earlier (Kimura et al 1964). The bones were not decalcified and a drill was used only to thin the bony capsule of the apex before opening it with a pointed needle. The specimens were embedded in Epon, bisected along the longitudinal axis of the modiolus and each turn was separated out and divided into smaller pieces with a razor blade. The modiolar part containing the spiral ganglion was separated from the organ of Corti at the osseous spiral lamina and the bone surrounding the neuronal masses was eliminated. The specimens were initially examined under the phase contrast microscope then cut with an LKB ultratome and photographed at 1000 to 30000  $\times$  magnification with a Siemens Elmiskop I. A total of 17 bones from 12 individuals with an age distribution of 9 months, 6, 7, 17, 31 (two specimens), 37, 42, 59, 65, 75 and 92 years were examined. None of these cases with the exception of the 59-year-old who had Meniere's disease had a history of

known otological complaints. Two specimens age 9 months and 37 years, were serially or semiserially sectioned and series of large composite electron micrographs were made. Each neuron was followed in sequence to a thickness of more than 50  $\mu$ m which was sufficient to include the width of the neurons.

## FINDINGS

A total of 971 human spiral ganglion cells was studied. In direct contrast to the findings in small animals, a majority of the neurons were unmyelinated. There were essentially two groups of neurons, large and small (Fig 1). Both myelinated and unmyelinated types were observed within each group. The cytological details of the large and small neurons differed, though a limited number of the small cells showed a cell organelle arrangement similar to the large ones.

In our study the large neurons formed 94% of the population. The perikarya were 22 to 34  $\mu$ m in diameter and 22 to 64  $\mu$ m in length. They were mostly encapsulated by a single sheath of satellite cells (Fig 2A).

## THE MIDDLE EAR GAS COMPOSITION IN AIR VENTILATED DOGS

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**Abstract** The middle ear gas composition has been examined in 5 air ventilated dogs under sodium thiopentone anaesthesia. The gas samples are obtained by trans-tympanic puncture and analysed by gas chromatography. The following mean  $\pm$  S.D. gas composition was obtained:  $N_2$  83.2  $\pm$  5.0,  $O_2$  1.1  $\pm$  2.2, and  $CO_2$  4.7  $\pm$  0.7.

Elner 1976 1977) In this communication we present the results of the examination of the MEG composition in air ventilated dogs performed by a micro-method recently described by us (Ostfeld et al. 1979).

The Middle Ear Gas (MEG) is an essential component for normal Middle Ear (ME) function. Its quantitative alteration may cause pathological conditions such as the atelectatic ear (Sadé & Berco 1976) or the overinflation syndrome (Tjernström 1977). Both situations are related to a decrease or increase in the total MEG pressure. Alterations in the relative composition of the MEG have focused little attention though recently Ingelstadi et al. (1975) and Sadé & Weisman (1977a 1977b) showed that changes in the concentration of the individual gases oxygen ( $O_2$ ) or carbon dioxide ( $CO_2$ ) may be etiologic for some pathologic conditions. Quantitative and qualitative change in MEG occur under general anaesthesia with nitrous oxide ( $N_2O$ ), the accumulation of which has been described as being responsible for lateralization of the graft during tympanoplastic surgery, neotympanum perforations and damage to the reconstructed ossicular chain (Thomsen et al. 1965; Paterson & Bartlett, 1976). Those observations suggested the need for knowledge of the MEG composition under normal conditions and study of the dynamics of gas exchange in the ME by diffusion across the ME mucosa.

Very few and incomplete data are available on the composition of MEG (Drettner 1975

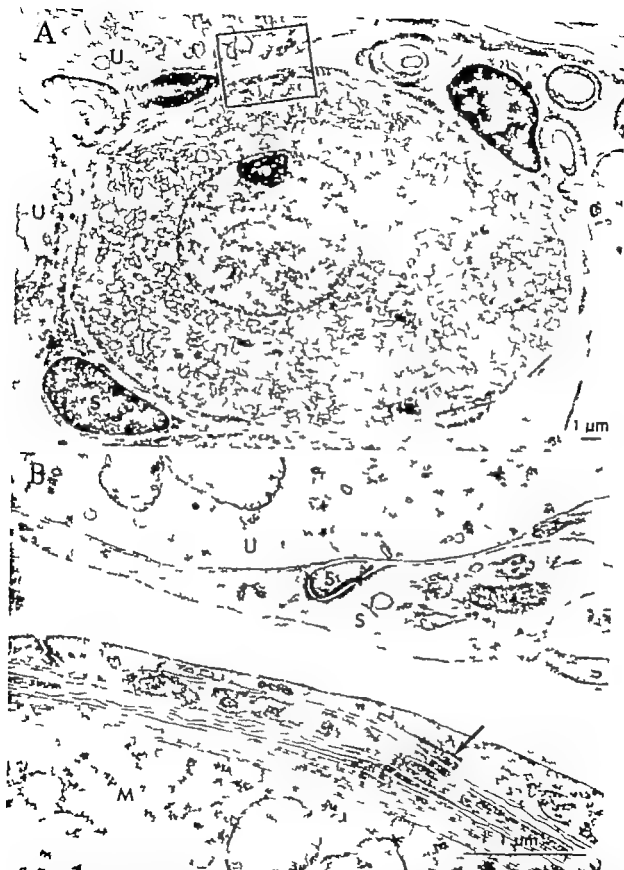
## MATERIAL AND METHODS

The MEG examinations were carried out on mongrel dogs. Sodium thiopentone i.v. was used for anaesthesia. During the procedure the dogs were ventilated with air by a Harvard pump through an endotracheal tube. The respiratory rate was kept at 16-18 respirations per minute.

The tympanic membrane (TM) was visualized through a window in the posteromedial part of the vertical portion of the cartilaginous external auditory conduct. This was performed by a retroauricular approach.

Accurate visualization was achieved by magnification of an operating microscope through an ear speculum introduced into the shortened ear canal. Only one ear was exposed during each experiment so that the head position was not changed and only healthy ears with non-perforated TM were included. Hamilton gaslight syringes were used for trans-tympanic MEG sampling. Leakage of MEG or inadvertent admixture with the environmental air was prevented by performing the sampling under a seal of 4-5 mm saline covering the TM.

Established Investigator of Chief Scientist's Bureau, Ministry of Health, Jerusalem.



**Fig. 3** (A) A large myelinated neuron showing cytoplasm filled with mitochondria, free ribosomes and rough endoplasmic reticulum. Mitochondria are edematous due to post mortem changes. Myelinated neurons are often difficult to determine under low magnification. Unmyelinated large neurons (U). Satellite cell (S).

(B) A higher magnification of the bracketed area in Fig. 3A showing myelinated (M) and unmyelinated (U) neurons. Note a stack of desmosomes (arrow) in the myelin sheath and desmosomes between two satellite cells (S).

## AUTONOMIC DYSFUNCTION ON THE AFFECTED SIDE IN MENIERE'S DISEASE

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**Abstract** For ascertaining the existence of autonomic dysfunction on the affected side as being the cause of Meniere's disease the mechohyl tests were conducted by means of conjunctival instillation in normal subjects as well as in patients with Meniere's disease labyrinthitis and vestibular neuritis. The rate at which significant moxa (more than 10%) appeared in the mechohyl test was 3.1% among normal subjects, whereas the rate among Meniere's disease patients on the affected side was significantly higher during the attack, quasi-attack and interval stages. The appearance rate among Meniere's disease patients on the unaffected side was not different from that in normal subjects during any of the three stages. None of the patients with labyrinthitis or vestibular neuritis showed significant moxa on the affected side alone in the mechohyl test. This indicates that abnormality of vestibular-autonomic reflex in itself does not cause positive reaction in the mechohyl test. Hence it can be concluded that the cause of Meniere's disease is related to the existence of autonomic dysfunction on the affected side.

Although it is generally believed that autonomic dysfunction is the cause of Meniere's disease (Williams 1965) little evidence clearly indicating the presence of such abnormalities in patients suffering from this condition has been available. Uemura et al (1972) examined the effects of mechohyl administered intramuscularly on the blood pressure of 20 patients with Meniere's disease. The positive rate i.e. showing a response of either sympathetic hyperreactivity or sympathetic hyporeactivity (Gellhorn & Redgate 1955 Redgate & Gellhorn, 1955) was found to be higher in these cases than in otitis media patients who complained of vertigo after ear surgery as well as in normal subjects. The difference was particularly significant during the acute stage of Meniere's disease in which vertigo or nys-

tagmus was present. However on the basis of this study we cannot explain the fact that most patients with Meniere's disease are affected unilaterally.

The pupils are innervated by the autonomic nervous system alone and they can react independently of each other. For these reasons we conducted mechohyl tests by means of conjunctival instillation to study the pupillary responses of patients with Meniere's disease and compared the results with those obtained from normal subjects as well as from patients suffering from labyrinthitis or vestibular neuritis.

### APPARATUS

The binocular infrared pupillograph we have devised was used (Fig. 1). As shown in the block diagram (Fig. 7) the light from the halogen lamp goes through an infrared filter and illuminates each of the patient's eyes separately. After passing through the objective lenses and a half-prism the images of the pupils enter the camera and the image intensifier simultaneously. The examiner can thus observe the pupils as displayed on the image intensifier. This equipment has the following characteristics. (1) Employment of an image tube allows visualizing and photographing of the pupil in dark infrared illumination. (2) Combined images of both eyes eliminating the space occupied by the bridge of the patient's nose can be obtained through the use of the prism and (3) the photographs can be taken while the patient is in a supine position. A motor drive



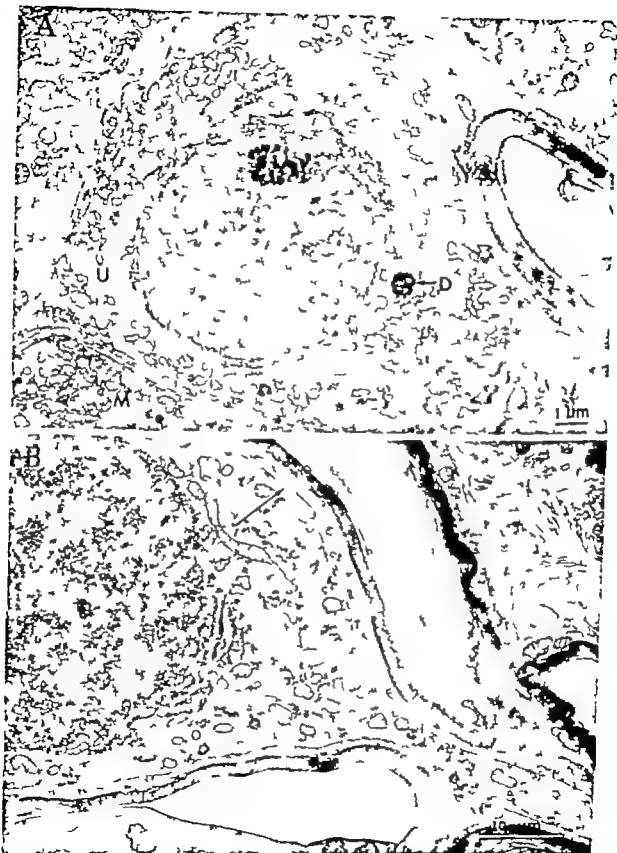


Fig. 4. (A) A small unmyelinated neuron (U) adjacent to the small myelinated neuron (MF) shows the filamentous cytoplasm. Mitochondria, free ribosomes and rough endoplasmic reticulum are relatively sparse. The nucleus is round and the nucleolar substance is loosely organized. Compare with the large neurons in Figs. 1 and 3.

Dense inclusion (D). (B) A small unmyelinated neuron showing a dendritic process. Its satellite sheath contains an unmyelinated nerve fiber (arrow). The dendritic process shows a distribution of cell organelles similar to that of the perikaryon and the neurofilaments are not obvious.

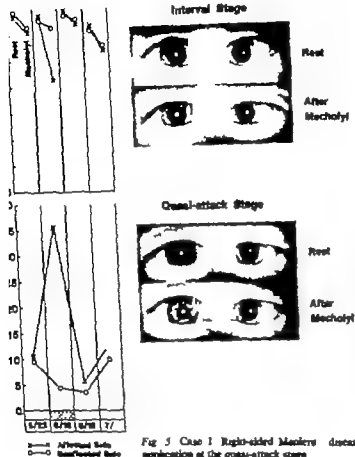


Fig 5 Case 1 Right-sided Meniere's disease showing right nystagmus after mechohyl application at the quasi-attack stage

None of the 4 patients with vestibular neuritis who could be tested within 2 weeks after onset showed pathological miotic of the affected side in the mechohyl tests, with one showing positive results on the normal side. The severity of the vertigo in the 4 cases was equivalent to that at the quasi-attack stage in Meniere's disease. In all 4 cases spontaneous nystagmus toward the normal side was present at the time of the testing and either no response or a severe canal paresis was revealed by the caloric test.

### Case Reports

#### Case 1

H.S., a 50-year-old male with Meniere's disease on the right side, was first seen on May

22, 1976. Two and a half years prior to the initial examination the patient had experienced attacks of rotary vertigo with tinnitus and hearing loss, both on the right side. On June 16, 1976 the tinnitus and hearing loss worsened, and spontaneous nystagmus toward the affected side appeared about an hour before the mechohyl test was started. He felt as if an attack were imminent but did not have rotary vertigo (quasi-attack stage). The mechohyl test revealed obvious miotic on the right side. However no difference between the pupillary diameters before and after mechohyl application was noted on the left side. Three mechohyl tests were conducted on this patient before and after the quasi-attack stage but none showed any pathological miotic on the affected side (Fig. 5).

multipolar. These neurons were fusiform or irregular in shape. The size of the peripheral processes (dendrites) was larger or almost equal to that of the axonal process. The myelin sheath when present was loosely organized and numbered up to 14 layers (Fig 5A-B). The cytological characteristics in both myelinated and unmyelinated small cells were similar. In the cytoplasm there were numerous neurofilaments in loose (Figs 4A-5B) and bundle forms which gave the impression of a paucity of cell organelles. The density of filaments varied and the filaments were less obvious in a few cells. Near the nucleus was an aggregate of vesicles and cisternae presumably Golgi network. Dense cored vesicles were not evident. One or two basal bodies were observed in a few neurons. The nuclei were round and were located in the center or slightly off-center. The nuclear chromatin substance was dispersed in a manner similar to that of the large neurons. However the nucleolar substance of the small neurons showed an irregular shape and loose arrangement instead of the round or oval compact or dense forms of the large neurons. Desmosomes puncta adhaerentia, were found between the sheath of the satellite cell and the plasmalemma of the perikaryon. The sheath often contained one to three unmyelinated nerve fibers close to the perikaryon plasmalemma (Fig 4B). The small neurons were randomly located however there was a tendency for them to be located near the nerve fibers of the osseous spiral lamina or at the periphery of the neuronal mass (Fig 1).

## DISCUSSION

From the present study as well as from our earlier ultrastructural studies of the human inner ear (Kimura et al 1964 1976) it has become increasingly clear that there are certain morphological differences in human ears in comparison to those of small animals. The spiral ganglion is a prime example. As

stated earlier the majority of animal spiral ganglion cells are myelinated. In our own study (Kimura, unpublished data) of rhesus and squirrel monkey inner ears the myelinated type was the most common. It is difficult to conceive why such differences or almost a reverse proportion exists in the human since neuronal function is expected to be the same. The possible significance may lie in conduction of neural impulses, that is the conduction rate in humans may be slower since the neurons lack myelin sheaths. It is well known that the unmyelinated nerve fibers have a much slower conduction velocity than myelinated fibers (Tasaki 1953). Other factors which would influence conduction along the perikarya are the extent of unmyelinated portions of the dendrites and axons as well as the resistance provided by the neural surface and cytoplasm all of which are greater in the human than in small animals.

No essential difference in morphology was noted in the satellite cell versus the Schwann cell and both of these cells are derived from the neural crest (Peters et al 1970). The presence or absence of myelin sheath does not seem to be related to neuronal size in the human since it was observed around both large and small neurons. Maturation is evidently not a factor related to myelogenesis in the rat (Ross & Burkell 1973). In man considerable individual variation is noted in the distribution of myelinated neurons however there is a slight tendency for more myelinated neurons to be present in older individuals. Unmyelinated neurons may become myelinated or simply the number of myelinated neurons increases due to a decrease in the number of unmyelinated neurons. The population of human spiral ganglion cells is known to decrease in aged individuals (Schuknecht 1964 Suga & Lindsay 1976 Otte et al 1978) with or without a decrease in the sensory cell population. The survival of neurons depends on environmental factors such as extracellular fluid

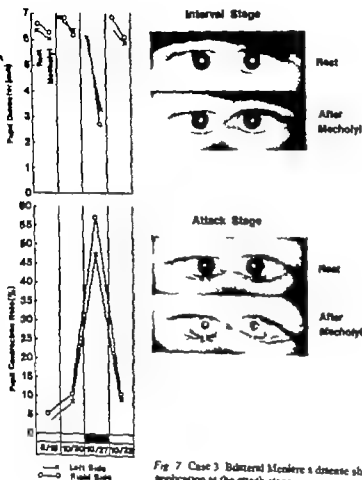


Fig 7 Case 3. Bilateral Meniere's disease showing anisocoria of both eyes after mechohyl application at the attack stage.

### Case 3

A M, a 39-year-old female with bilateral Meniere's disease, was first seen on July 31, 1976. Three weeks prior to her first visit, the patient had experienced a vertiginous attack with bilateral tinnitus and a sense of blocking of the ears. An attack of clockwise turning sensation accompanied by nausea began around 6:30 p.m. on October 27, 1976. Spontaneous nystagmus toward the right side was observed. The mechohyl test conducted during the attack showed remarkable miosis in both eyes. The three tests undertaken at the interval stage, however, did not indicate any abnormality (Fig. 7).

### DISCUSSION

The mechohyl test by means of conjunctival instillation has been used to determine whether a large pupil is caused by sympathetic overactivity or by parasympathetic paralysis. If the test shows significant miosis, the case is diagnosed as the latter. It is also said that miosis is more pronounced after a postganglionic than a preganglionic lesion. This phenomenon may be explained by supersensitivity to acetylcholine or other related agents, such as mechohyl, developed at the area of the neuroeffector junction after section of a parasympathetic nerve. The development of this supersensitivity does not occur immediately after

multipolar. These neurons were fusiform or irregular in shape. The size of the peripheral processes (dendrites) was larger or almost equal to that of the axonal process. The myelin sheath when present was loosely organized and numbered up to 14 layers (Fig. 5A, B). The cytological characteristics in both myelinated and unmyelinated small cells were similar. In the cytoplasm there were numerous neurofilaments in loose (Figs. 4A, 5B) and bundle forms which gave the impression of a paucity of cell organelles. The density of filaments varied and the filaments were less obvious in a few cells. Near the nucleus was an aggregate of vesicles and cisternae, presumably Golgi network. Dense cored vesicles were not evident. One or two basal bodies were observed in a few neurons. The nuclei were round and were located in the center or slightly off-center. The nuclear chromatin substance was dispersed in a manner similar to that of the large neurons. However, the nucleolar substance of the small neurons showed an irregular shape and loose arrangement instead of the round or oval compact or dense forms of the large neurons. Desmosomes, puncta adherentia, were found between the sheath of the satellite cell and the plasmalemma of the perikaryon. The sheath often contained one to three unmyelinated nerve fibers close to the perikaryon plasmalemma (Fig. 4B). The small neurons were randomly located; however, there was a tendency for them to be located near the nerve fibers of the osseous spiral lamina or at the periphery of the neuronal mass (Fig. 1).

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Table 1 Radiographical size of the mastoid air cell system

R=right ear L=left ear B=bilateral ears

|                                  | Male            |             |                                    |      | Female          |           |                                    |      |
|----------------------------------|-----------------|-------------|------------------------------------|------|-----------------|-----------|------------------------------------|------|
|                                  | No. of subjects | No. of ears | Mean $\pm$ S.E. (cm <sup>2</sup> ) | S.D. | No. of subjects | N of ears | Mean $\pm$ S.E. (cm <sup>2</sup> ) | S.D. |
| Meniere's disease                | 16              | 17          | 11.63 $\pm$ 1.29                   | 5.31 | 11              | 11        | 8.20 $\pm$ 1.10                    | 3.66 |
| Other sensorineural hearing loss | 16              | 16 (R)      | 13.81 $\pm$ 1.22                   | 4.86 | 11              | 11 (R)    | 10.97 $\pm$ 1.27                   | 4.11 |
|                                  |                 | 16 (L)      | 13.47 $\pm$ 1.08                   | 4.31 |                 | 11 (L)    | 10.35 $\pm$ 1.08                   | 3.58 |
|                                  |                 | 32 (B)      | 13.64 $\pm$ 0.80                   | 4.52 |                 | 22 (B)    | 10.66 $\pm$ 0.82                   | 3.83 |
| Clinically healthy               | 13              | 13 (R)      | 13.29 $\pm$ 1.60                   | 5.76 | 12              | 12 (R)    | 10.12 $\pm$ 1.05                   | 4.43 |
|                                  |                 | 13 (L)      | 13.42 $\pm$ 1.54                   | 5.46 |                 | 12 (L)    | 10.13 $\pm$ 1.01                   | 4.52 |
|                                  |                 | 26 (B)      | 13.36 $\pm$ 1.09                   | 5.54 |                 | 24 (B)    | 10.12 $\pm$ 0.72                   | 4.50 |

ear healthy ( $P < 0.05$ ). No statistical differences were seen between male and female of Meniere's disease, other sensorineural hearing loss and clinically healthy ears ( $P < 0.05$ ).

### DISCUSSION

The correlation between poor pneumatization of the mastoid and chronic otitis media has been reported by several authors. Diamant (1940) suggested that the pneumatization of the temporal bone may be caused mainly by hereditary factors and "chronic otitis media" which tends to occur in such a situation. Tumarkin (1957) suggested that otitis media in intrauterine life or early childhood can disturb pneumatization of the temporal bone. Kollbova et al. (1972) planimetrically investigated the mastoid air cell system of the children who had had acute otitis media on at least one occasion and then compared them with those of normal children (Rubensohn, 1965). They found that the adequate treatment of the infection decreased retardation of the pneumatization of the temporal bones.

Meanwhile, Holmquist (1969) reported a correlation between the size of the mastoid pneumatization and the tubal function which is maintained to be closely associated with chronic otitis media. However, Andréasson (1976) insisted that the functional volume of the mastoid measured by physiological pres-

surre technique is much more correlated with the tubal function because the mucosal damage of the middle ear and the mastoid can not be estimated correctly by measuring radiographical area.

The relationship between Meniere's disease and the dysfunction of the auditory tube was reported by Tumarkin (1966) and Lall (1969). According to Tumarkin, the insertion of ventilating tube through the tympanic membrane is a rational treatment, on the basis of intermittent tubal obstruction of Meniere's disease. However, Cinnamon (1975) disagreed with this theory.

In our study, we could not find any statistical differences of the mastoid pneumatization among Meniere's disease, other sensorineural hearing loss and clinically healthy ears. This may at least mean that the infection of the middle ear in childhood is not the main aetiological factor for Meniere's disease. Moreover, the size of the mastoid air cell system does not represent the size of the periaqueductal pneumatization with reference to the report of Wilbrand et al. (1978).

The mean value of the radiographical area of the mastoid air cell system in Meniere's disease was less than those in other sensorineural hearing loss and in the clinically healthy ears. However, the differences were not statistically significant.

We cannot say whether the dysfunction of the auditory tube may be one of the aetiological

the range of individual differences. These figures are strikingly similar to those of the cat before section of the efferent nerve fibers (Spoendlin 1974) and our own study on the normal squirrel monkey (Kimura, 1978). Thus the same distribution pattern holds in the human as in animals; however, termination of dendritic processes of the neurons remains to be determined in the human.

## ZUSAMMENFASSUNG

Elektronenmikroskopische Untersuchungen des menschlichen Ganglion spirale wurden in 17 Ohren von 12 Individuen im Alter von 9 Monaten bis zu 97 Jahren durchgeführt. Zwei Arten von Neuronen und zwar große und kleine mit bestimmten cytologischen Merkmalen wurden gefunden. Beide Arten waren sowohl myelinisiert als auch unmyelinisiert. Die Mehrzahl war unmyelinisiert (94%). Der Anteil myelinisierter Neurone variierte stark von Individuum zu Individuum, doch eine Zunahme bei alten Personen wurde gefunden. Der größte Anteil war 28% bei einer Person im Alter von 75 Jahren. Sechs Prozent etwa waren kleine Neurone. Ihr Cytoplasma enthielt viele Neurofilamente sowohl in der myelinisierten als auch in der unmyelinisierten Art. Die vorliegende Untersuchung sagt nichts betreffend die funktionelle Bedeutung der Myelinisierung von Spiralganglionzellen aus.

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cifically selected for this study because they have been demonstrated to inhibit abducens motoneurons and because of their important role in nystagmus generation.

## MATERIALS AND METHODS

Twelve cats weighing from 2.0 to 3.5 kg. were used. Animals were anesthetized with halothane in oxygen through a cuffed endotracheal tube and mounted in a stereotaxic instrument. The animals were put in a supine position and using a ventral approach one labyrinth was surgically destroyed. A bipolar stimulating electrode consisting of fine silver wires insulated except for their Ag-AgCl spherical tips was placed in the destroyed labyrinth. The animals were then brought to a prone position with bodies comfortably supported on a plastic foam bed. The ipsilateral abducens nerve and medial rectus division of the oculomotor nerve were detached from their innervating muscles by an orbital approach and mounted on fine Ag-AgCl hook electrodes. A small portion of the occipital bone was removed and the mid line cerebellum was aspirated to expose the floor of the fourth ventricle enabling a micro-electrode to be inserted into the brain stem under visual control with the assistance of an operating microscope. A pair of stainless steel screw electrodes were placed in the skull over the anterior sigmoid and posterior lateral cortices for recording EEG. Bilateral pneumothorax was made to reduce movement of the brain stem. A catheter was inserted into the femoral artery and the arterial blood pressure and heart rate were continuously monitored on a large dial meter and intermittently on a strip chart recorder. Rectal temperature was kept at about 37°C by a heating pad. All wound edges and pressure points were infiltrated with 1% procaine. The halothane was then discontinued and the animal was immobilized by intravenous administration of gallamine triethiodide (Flaxedil) under artificial respiration with room air. Recording was not started until

at least two h after discontinuation of halothane anesthesia.

Local anesthesia was maintained by repeated administration of procaine throughout the experiment. The animal was awake since alertness is necessary for the production of regular nystagmus which was the control condition in this study. The effectiveness of the local anesthetic in controlling pain was carefully checked by observing changes of the heart rate (Lenox 1970) blood pressure (Kryzhanovskiy 1976 Lenox 1970 Wolf & Hardy 1941) pupillary size (Kryzhanovskiy 1976, Maeda et al 1972 Römer 1968) and EEG pattern (Maeda et al 1972) at rest. The animals were considered to be comfortable when the heart rate was regular in the range from 140 to 180/min blood pressure stable in the range of from 100 to 160 mmHg, pupil either in slit-like or mid-dilation and EEG showed 5-7/sec activity with infrequent 3 to 4/sec spindles. Furthermore appreciable changes on these parameters were not induced by tactile stimulation of wound edges. On a few occasions when the local anesthetic was not renewed in time heart rate rose pulse pressure widened and blood pressure rose or became fluctuating and the pupil became larger. Within seconds after injection of procaine into wound edges these symptoms returned to normal.

After recovery from halothane anesthesia, most animals showed alternating rhythmic activity of the abducens and medial rectus nerves. These are the neural counterparts of regular vestibular nystagmus with the quick phase directed toward the side of the intact labyrinth. In those instances where these patterns of nerve discharges were not regular or were replaced by tonic discharges 5 mg/kg of 1 amphetamine sulphate was given intravenously (Bradley 1968). In order to reverse the direction of nystagmus high frequency (usually 200/sec) electrical stimulation using 0.1 msec pulses was applied to the vestibular nerve on the labyrinthectomized side.

Glass micropipettes filled with 2M NaCl so-



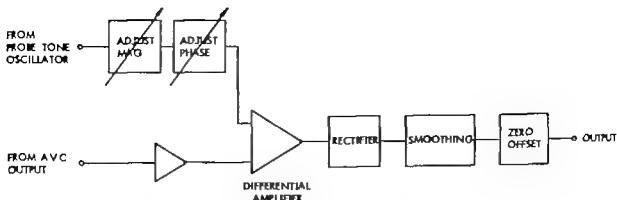


Fig 1 Additional circuit for admittance modulus measurement.

It is not sufficient merely to obtain the amplitude of this signal when dealing with measurements of eardrum admittance. It is usually also necessary to compensate for the ear canal volume between the probe and the eardrum so that measurements are independent of probe placement. When conductance and susceptance are available separately the conductance and susceptance of the ear canal mutually obtained by tympanometry

can simply be subtracted arithmetically from the conductance and susceptance measure during reflex contraction. However it is not valid to do this in a *scalar* manner for the admittance modulus. The errors involved in doing so for a probe tone frequency of 22 Hz are not normally large and many instrument manufacturers accept this error. However for the probe tone frequency of 660 Hz which is additionally available on the

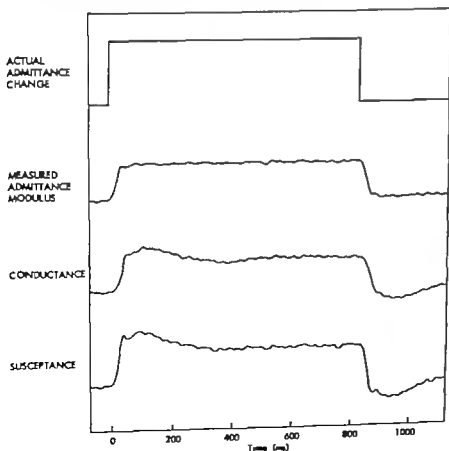


Fig 2 Transient characteristics of admittance modulus, conductance and susceptance measures. The top trace indicates step changes in admittance whilst the three lower traces show responses of the instrumentation to this change. Note that only the conductance and susceptance traces exhibit overshoot.

n exactly opposite fashion. Similar results can be seen in Fig. 6.

#### *The early stages of anesthesia*

The burst activity of BINs was clearly changed even in the early stage of nitrous oxide anesthesia. In all instances the changes were closely related to changes in the discharge patterns of the contralateral abducens and medial rectus nerves and in the EEG. The most common change was a decrease in the maximum firing rate of each burst (28 of 30 neurons). In addition 21 of 28 BINs exhibited an increase in the burst duration and a decrease in the number of bursts per sec.

The response of a typical neuron is shown in Fig. 3. The firing rate of this neuron (row 2 in inset figures) was recorded simultaneously with EEG (row 1), contralateral abducens nerve discharge (row 3) and blood pressure (row 4). The time course of changes in the burst activity was shown by successive measurements in 1 sec intervals of the maximum firing rate, the burst duration and the number of bursts. In the unanesthetized control state characterized by low voltage fast waves in the EEG this neuron showed a high maximum firing rate of 600 spikes/sec. This rate started to decrease gradually about 10 sec after the beginning of anesthesia. This tendency to decrease firing rate (A) lasted during anesthesia and was accompanied by a slowing and amplitude increase of the EEG. The duration of each burst increased approximately linearly from 120 to 240 msec (B). In contrast, the number of bursts gradually decreased from 4/sec to 1/sec (C). BINs varied in their resistance to the effects of anesthesia; some were completely silenced after 20 sec, others continued to show some bursting activity 240 sec after anesthesia onset.

Close inspection of abducens nerve discharge traces (Fig. 3, row 3 in inset figures) shows that in the control record there is an abrupt cessation followed by a short term complete suppression of the nerve discharge at the quick phase. This suppression was grad-

ually replaced during anesthesia by a relatively long-lasting, weak suppression while the slow phase discharges were gradually prolonged and reduced in number and amplitude. The envelope of the abducens discharge changed from a distinctly triangular shape in the control record to a much more rounded shape in anesthesia, corresponding to a decline in quick phase velocity.

It should be noted here that, apart from the above changes, no marked or consistent change in blood pressure was observed. A level around the control value was maintained (row 4).

The changes in a given BIN in its firing rate, burst duration and number of bursts described above were not invariably related to each other. Three neurons exhibited a decrease in firing rate and an increase in burst duration without any clear change in the number of bursts. In three other neurons only a decrease in the BIN firing rate was observed. In the remaining two neurons, both from the same animal, the number of bursts per sec decreased during anesthesia, but the maximum firing rate increased. The burst duration remained fairly constant. In one cell there was an increase in the number of bursts whereas the maximum firing rate decreased substantially. In a few neurons the maximum firing rate increased and was accompanied by the usual increase in the burst duration and decrease in the number of bursts/second.

Yet even in the unusually behaving BINs described in the paragraph above there was still a precise relationship between certain discharge characteristics of these single neurons and the abducens nerve discharge. Thus the increased firing rate of BINs was accompanied by an increased sharpening of the end of abducens slow phase discharge. The decrease in the number of bursts/sec was accompanied by a decrease in the number of nystagmus beats.

The time to the first detectable change in either the maximum firing rate, duration or number of bursts from the onset of anesthesia was measured for each BIN neuron and ran-

# PROBLEMS AND PITFALLS IN OTOTOXICITY TESTING

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**Abstract** A local anaesthetic drug under development, intended for use in ear surgery was tested with regard to adverse, ototoxic effects. Inner ear damage was not obvious. However in the guinea pig a massive formation of new bone occurred in the middle ear (bullae tympanica) within 5 weeks after a single local administration of the drug.

drug under development and of whether or not it can exert ototoxic effects in the guinea pig when instilled into the middle ear

## MATERIAL AND METHODS

Since the introduction of the concept of ototoxicity in the early 1950's research has often been focused on the effects on the labyrinth following the administration of aminoglycoside antibiotics given either systemically (Wersäll & Hawkins 1962 Wersäll et al 1973 review Hawkins 1976) or by local instillation into the middle ear (Wersäll et al 1969). During the 1960's and 1970's both environmental poisons (Falk et al 1974 Anniko & Sarkady 1978) and potent diuretics (Brummett et al 1977 Anniko 1978) have been shown to exert ototoxic effects under certain conditions.

In the treatment of middle ear disease drugs with anaesthetic or antimicrobial effects have often been used locally e.g. Xylocain® injected subcutaneously or washing with solutions iodine solutions etc. To simplify the use of local anaesthetics in polyclinical work drugs having a rapid skin penetration have been developed which thus give complete anaesthesia following topical application. This property is of great value for instance in the treatment of patients needing transmyringal drainage. A prerequisite is however that such a drug is atoxic with regard to the middle ear and inner ear structures.

The present study is an analysis of one such

The present series consisted of 30 healthy young guinea pigs (200 g) with a preserved Preyer's reflex and a normal tympanic membrane as observed in the operating microscope. The animals were free from clinical signs of vestibular dysfunction. They were divided into three groups each animal in the same group receiving 0.15–0.20 ml of either solution A, B or C respectively. The study

Table I Table illustrating the composition of the test solutions A, B and C

Solutions A and B are patented by Astra Pharmaceuticals AB, Sweden

|                   |          |
|-------------------|----------|
| <b>Solution A</b> |          |
| 100 ml contains   |          |
| Isopropanol       | 50 g     |
| Glycerine         | 13 g     |
| Conc. acetic acid | 0.1 g    |
| Water             | 28 g     |
| <b>Solution B</b> |          |
| 100 ml contains   |          |
| Ketocaine         | 10 g     |
| Isopropanol       | 43.1 g   |
| Glycerine         | 11.6 g   |
| Conc. acetic acid | 11.1 g   |
| Water             | 25.2 g   |
| <b>Solution C</b> |          |
| Phys. saline      |          |
| NaCl              | 155 mmol |
| Distilled water   | 1 000 ml |

contralateral abducens nerve discharge, and by a progressive slowing of EEG

The results indicate that BINs exert the powerful inhibitory effect on contralateral abducens motoneurons. Even small changes in the firing of only one BIN were clearly reflected in the discharge pattern of the entire abducens nerve. This powerful control could arise from each BIN branching profusely and inhibiting many of the motoneurons (Hikosaka & Kawakami, 1977; Hikosaka, et al. 1978b) or the uniform discharge pattern of all BINs (Hikosaka & Kawakami, 1977) or both.

The activity of most BINs was clearly suppressed by nitrous oxide. This result is in contrast to those in studies of other central neurons. At the same concentration of anesthesia is used here, the spontaneous firing rate of spinal dorsal horn neurons was only moderately reduced in a brief initial period (de Jong et al. 1970) and no consistent change was observed in the resting firing rate of thalamorelay neurons (Mon et al. 1977). The depressant effect of nitrous oxide on BINs was not due to a decrease in blood pressure which has been reported to reduce the spontaneous activity of a class of brain stem neurons (Baust & Niemczyk, 1963) since no clear or consistent change in blood pressure was induced by nitrous oxide in the present study.

The mechanism of nitrous oxide anesthesia is still not known. Recently it has been suggested that it releases or potentiates endogenous opiates (Berkowitz et al. 1976) although other evidence puts this suggestion in some doubt (Smith et al., 1978).

Early in anesthesia, most BINs showed a synchronous decrease in maximal firing rate, increase in burst duration and decrease in the number of bursts/sec. However, a few BINs did not show this typical pattern. In one instance an increase in the number of bursts was found, whereas the maximum firing rate decreased substantially. In a few neurons, the maximum firing rate increased and was accompanied by the usual increase of the burst duration and a decrease in the number of bursts/second. This

dissociation in changes of the discharge parameters of BINs suggests that those parameters might be controlled by different neuronal elements which are simultaneously affected in most instances by nitrous oxide but independent in exceptional cases.

In the later stages of anesthesia, the firing pattern of typical BINs was changed from burst to tonic firing which was finally suppressed completely. At the same time abrupt changes in activities of the eye motor nerves which indicate presence of the quick phase disappeared, and the discharge of the agonist nerve became tonic and the antagonist nerve became silent. Similar changes were observed in an additional two animals in which no labyrinthectomy was performed and horizontal vestibular nystagmus was elicited by sinusoidal oscillation of the turntable. During nitrous oxide anesthesia, firing of BINs gradually disappeared and the abrupt changes in discharges of the opposing eye motor nerves were replaced by reciprocal spindle-like discharge patterns in phase with turntable oscillation. These results indicate that the neuronal mechanism generating the quick phase is more susceptible to anesthesia than that of the slow phase. Further, these results indicate that the primary and secondary vestibular neurons and ocular motoneurons were relatively unaffected by the anesthesia to the extent that they were still capable of faithfully responding to the angular acceleration stimulation. According to Janke et al. (1969) the quick phase of the vestibular nystagmus is selectively abolished by fentanyl, a morphine-like substance. They speculated that the drug suppressed the quick phase centrally, leaving the basic three neuron vestibulo-ocular pathway intact and that the possible site of the action of the drug is the reticular formation. Our results at the neuronal level support this speculation.

In the depths of anesthesia and at a time when BINs were silent, the abducens nerve showed tonic activity while the medial rectus nerve was virtually silent (Fig. 6A). As anesthesia deepened still further the abducens



## AN UNCOMMON APUDOMA A FUNCTIONAL CHEMODECTOMA OF THE LARYNX

### *Report of a Case and Review of the Literature*

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**Abstract** The authors report a case of laryngeal chemodectoma in a 53-year-old man who died from general anaesthesia with laryngeal and subcutaneous metastases. This functional neoplasm secreted calcitonin related by fluoroscence and biochemical tests and also adrenaline-like substances. Its cells looked like light chief cells of the human carotid body: they showed secondary granules and lysosomal formations. Only twelve cases had been previously reported. The laryngeal glomus and more occasionally the laryngeal glomus were the seat of these tumours which started with an equal frequency in both sexes. A sinusoidal vein often the unique symptom noticed during the several months or years preceding the first intubation of this slow growing neoplasm. Nevertheless among the chemodectomas of the head and the neck region those arising in the larynx had the highest evidence of malignant evolution. Five of the patients presented general dissemination, two had lymphatic metastases and one local recurrence. The ultra-structural features and sometimes the merely detected functional activity of these chemodectomas are those of paragangliomas whose cells might originate from the cephalic crest of the neural crest.

Chemodectomas or paragangliomas are uncommon tumours of the larynx. They occur in the superior laryngeal glomus located in the supraglottic larynx or in the inferior one situated between the cricoid cartilage and the first tracheal ring (Lawson & Zak, 1974). A hoarseness, a dysphagia and/or a cervical pain of several months to years duration preceded the discovery of these slow growth neoplasms. Unfortunately some of the chemodectomas are considered as epidermoid carcinomas. In this paper we describe such

a case whose issue was fatal. Speaking about 30 similar cases previously reported we should like to study the anatomic-clinical ultra-structural and functional features of laryngeal chemodectomas.

### CASE REPORT

#### *Clinical history*

In 1973 a 53-year-old man was seen by his doctor for subcutaneous thoracic nodes. At this time neither investigation nor therapy were prescribed. A few months later in February 1974 a hoarseness appeared. An epidermoid carcinoma was diagnosed after a laryngeal biopsy in July 1974. Then a laryngo-epiglottectomy and a bilateral cervical lymphadenectomy were performed. The left pyriform fossa was filled up with a 2.5x2x2 cm vegetant neoplasm which was destroying the homolateral false cord. The light microscopic examination confirmed the diagnosis of low differentiated epidermoid carcinoma invading two cervical lymph nodes. A post-operative radiotherapy was delivered on the two supra clavicle areas (5000 and 6000 rads) and on the nasopharyngeal field (5000 rads). In September 1974 multiple painful subcutaneous nodes were scattered all over the thorax the abdomen and the hips. Several nodes were removed to relieve the patient and with a view to making a light and electron microscopic study.



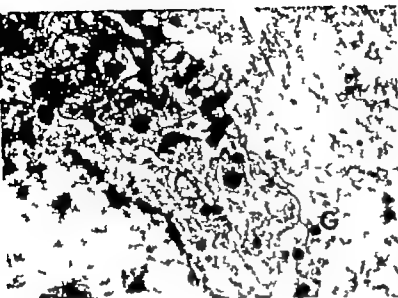


Fig 5 Typical secretory granules (G) were observed between the stromal collagen fibers and in the cytoplasm of tumour near by fibroblasts (I)  $\times 8600$ .

excretion gave the following results. adrenaline 135  $\mu\text{g}/24$  h and noradrenaline 390  $\mu\text{g}/24$  h (normal value adrenaline 4  $\pm$  8  $\mu\text{g}/24$  h and noradrenaline 25  $\pm$  50  $\mu\text{g}/24$  h). There was no variation of VMA (11.7 mg/24 h). Seventeen ceto and 17 OH steroids 5 H.L.A. calcemia and phosphoremia were unchanged.

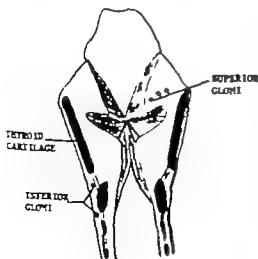


Fig 6 Topography of the laryngeal glomus.

## CONCLUSION

The laryngeal tumour was a functional chemodectoma with lymphatic and subcutaneous metastases.

## DISCUSSION

Thirty cases of laryngeal chemodectomas have already been reported (Table I). This neoplasm has an equal sex incidence. 15 cases have been discovered in men as well as in women with a maximum of frequency during the 5th decade of life. But two chemodectomas were individualized before the age of 20 in two young men, respectively 14 and 19 years old (Martinson, 1967; Ishida et al, 1971) and in four patients over 60 (Hooper, 1972; Tobin and Harris, 1972; Zachariah & Shah, 1972; Hohbach & Mootz, 1978). Most of these tumours arose from the pair of superior laryngeal glomus whose diameter ranged from 1 to 3 mm. According to Lawson & Zak (1974) these formations are located in the upper and anterior third of the ventricular fold adjacent to the superior margin of the thyroid cartilage. Somewhat





network and showed in one case only a central area of superficial ulceration (Adlington & Woodhouse 1977). On cut sections the fleshy soft tumour tissue was homogenous or impaired by little necrotic foci.

It is interesting to point out that among the chemodectomas of the head and neck region those arising in the larynx have the highest incidence of malignant. While 18 patients had no recurrence after radical surgery five others died with widespread dissemination in spite of surgery, radiotherapy and/or chemotherapy (Adlington and Woodhouse 1977, Hooper 1977, Zachariah & Shah 1977, Personal observation 1979). A local recurrence was well treated by excision (Wockel et al. 1964) and two patients bore lymphatic and/or subcutaneous metastases at the time of the operation (Vetters & Toner 1970, Fraidooni 1976). The diagnosis of this uncommon neoplasm was often difficult. An epidermoid carcinoma was suspected three times (Hooper 1972, Fraidooni 1976, Personal observation 1979). A cylindroma, a parathyroid adenoma and hemangiomas were also identified (Andrews 1955, Gignoux et al. 1964, Azevedo-Gamas & Gloor 1968). But the notion of the slow growth, the radiation resistance of the tumour and a new histological analysis of the specimens should enable a rectification of the diagnosis.

In effect the structure of these chemodectomas was quite invariable with its nets, cords or trabeculae of cells and abundant hyalin stroma containing numerous vascular channels. All these tumours presented the same cellular features as revealed by electron microscopic study (Vetters & Toner 1970, Adlington & Woodhouse 1972, Piquet et al. 1976, Hohlbach & Mootz, 1978, Personal observation). While the sustentacular cells and the nerve endings frequently found in the other chemodectomas were missing, the cells of these neoplasms were similar to the light chief cells of the human carotid body. They had a lucent cytoplasm with uniform spherical granules. The organelles

which play an important part in the secretory process, the Golgi complexes, the microtubules, the filaments and the endoplasmic reticulum surrounded the granules and the lysosomal formations. The presence of microvilli and desmosomes is a further argument for the epithelial nature of the tumour cells which with their granules could secrete. But that functional activity of laryngeal chemodectomas was rarely proved. Only Vetters & Toner (1970) detected catecholamines in their study. The Falck test was positive and noradrenaline was extracted at a concentration of 0.145 µg/g. Our observation gives a typical example of functional chemodectoma producing calcitonin. Besides the unchanged VMA and the high values of the catecholamines obtained by the fluorimetric method are perhaps suggestive of "catecholamine like" secretion. All these data enable us to classify these chemodectomas in the apudoma group. These apudomas are tumours whose cells derive from neural crest elements belonging to the APUD system (APUD: Amine Content/or Amine Precursor Uptake and Decarboxylation) described by Pearse (1969, 1974). The cells forming the superior and inferior laryngeal glomus would come from the cephalic portion of the neural crest and would go along the X and the vagospinal nerves (Kissel et al. 1976, Perrin et al. 1978). So their tumours as well as the other apudomas are rich in granules of neuro-secretory type and can secrete amine or amine precursor as in our observation.

## RÉSUMÉ

Les auteurs rapportent un cas de chimodectome laryngé chez un homme de 53 ans décédé dans un tableau de détérioration générale avec métastases ganglionnaires lymphatiques et sous-cutanées. Ce néoplasme fonctionnel sécrétait un taux élevé de calcitonine ainsi qu'en témoignent les dosages biochimiques et l'étude en immunofluorescence. Il s'agissait aussi, semble-t-il, des substances proches de l'adrénaline. Ses cellules qui ressemblaient aux cellules principales du glomus carotidien humain contrastaient des grains de type neurosecretor et des formations lysosomiales. Treize cas simili-

awaits further analysis. The initial reaction post injection giving symptoms of vestibular dysfunction has no morphological grounds and is a reversible phenomenon. Similar symptoms have been observed in clinical work when Xylocain® has accidentally been instilled into the middle ear (e.g. due to a perforation of the ear drum). Though these symptoms may last for a considerable time they are reversible leading to complete recovery.

The bulla tympanica of the guinea pig is known to be very sensitive to irritant agents and it is possible that such a massive formation of new bone during so short a time—previously not reported in the literature—may be restricted to this species.

When testing new drugs for the treatment of ear disease(s) it must be taken into consideration that they may primarily be relatively atoxic to inner ear hair cells yet exert considerable adverse effects on other adjacent structures thus impairing hearing *in toto*. Secondary changes in the labyrinth cannot be excluded after a long interval.

### ACKNOWLEDGEMENT

This work was supported by grants from Karolinska Institutet and The Swedish Medical Research Council (grant no. 12X 720).

### ZUSAMMENFASSUNG

Ein neues lokalanästhetisches Präparat, das jetzt für die Ohrenchirurgie entwickelt wird, ist mit Rücksicht auf die ototoxische Effekte studiert. Keine Innenohrschäden wurden gefunden. Aber im Mittelohr des Meerschweinchens entwickelte sich nach 3 Wochen eine massive Knochenstruktur nach einer einzelnen Verwendung des Präparates.

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Table 1 Data on five patients having dyspraxia

| Patient   | Age (years) | Symptoms and findings apart from dyspraxia            | Diagnosis                                   | Duration of speech disorders |
|-----------|-------------|---|---|------------------------------|
| 1 (A. S.) | 67          | Asymptomatic hypertension + extrapyramidal components | Multifocal, vasc. deg. of CNS               | 2 years                      |
| 2 (E. S.) | 57          | Inability to voluntarily activate cranial nerves      | Multifocal degeneration process in CNS ALS? | 2 years                      |
| 3 (B. K.) | 37          | Right sided hemiparesis                               | Left-sided anterotubal cerebral infarction  | 1 month                      |
| 4 (R. E.) | 68          | Difficulties in memory                                | Presenile dementia                          | 6 months                     |
| 5 (N. J.) | 4           | Right sided hemiparesis                               | Subarachnoidal bleeding, aneurysm           | months                       |

Conventional electronystagmographic equipment (Henriksson et al. 1967, Henriksson et al. 1972) was used. The methods in brief were as follows. Horizontal eye movements were recorded binocularly and vertical eye movements from each eye separately with a 4-channel DC-coupled pen-recorded (Mimograph 81 Siemens-Elema, Stockholm, Sweden) with an upper cut-off frequency of 15 Hz and an infinite time constant. Commercial electrodes (Medicotest®) with jelly were used. The subjects were examined with the respect to:

1. Command eye movements of 20°
2. Pursuit eye movements with a constant target velocity of 20°/s and with a pendular target.
3. Optokinetic nystagmus in a wholefield optokinetic drum accelerating from a velocity of 0° to 60°/s in 15 s.
4. Vestibular nystagmus was studied with open eyes in darkness (a) induced by caloric stimulation with water of 30°C and 44°C for 20° and (b) induced by angular accelerations of 120°/s<sup>2</sup> for 1 s.

## RESULTS

### 1. Command eye movements

The voluntary saccades were examined and compared with those of the normals (Fig. 1) with respect to the following parameters:

1. Accuracy. In four of the five patients there was a pronounced undershoot of the

command eye movements which were each performed as two to four smaller saccades. In one of the patients (No. 4) there were also overshoots by the eyes passing the target by five to eight degrees.

2. Fixation periods. The time intervals between the initiation of saccades of different directions were also studied. In four of the five patients these periods were very irregular while in one case (no. 2) these periods were significantly prolonged.

3. Velocity of saccades. The velocity of the saccades was also examined but did not show any differences from the normal subjects.

### 2. Following eye movements

Following eye movements were normal in patients 3 and 5 and a slight disturbance was seen in patient 1. Gross abnormalities with irregular disruptions of the smooth eye movements by saccades were found in patients 3 and 4 (Fig. 2). Four of the normal subjects presented completely smooth eye movements while one of these subjects showed a slight irregularity in his tracking pattern.

### 3. Optokinetic eye movements

The optokinetic nystagmus was within normal limits in all patients (Fig. 3). The fast and slow phases were consistently regular in both directions. The amplitudes were however smaller and the frequency higher in

awaits further analysis. The initial reaction post injection giving symptoms of vestibular dysfunction has no morphological grounds and is a reversible phenomenon. Similar symptoms have been observed in clinical work when Xylocain® has accidentally been instilled into the middle ear (e.g. due to a perforation of the ear drum). Though these symptoms may last for a considerable time, they are reversible, leading to complete recovery.

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### ZUSAMMENFASSUNG

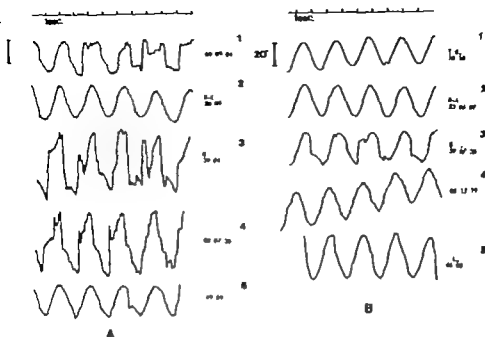
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2 The following eye movements with pendular saccades. (A) patients with dyspraxia, (B) healthy controls

strotatory and the caloric nystagmus were ak and of larger amplitude than in nor ds.

Three of the patients showed disturbances pursuit eye movements. These three tents had a pronounced increase in the plitude of the caloric nystagmus in rela- to the other patients and also when mpared to normal subjects

## DISCUSSION

### *A comparison between features of speech eye movements*

he pronounced relationship between speech isorder of the dyspraxic type and distur- nces in execution of command eye move- ments has not been previously described

In the highest centers speech would seem o be processed in three different stages a) Development of purpose (b) integrated planning; and (c) programming of the execu- tion of speech (Darley et al 1975)

Pure dyspraxia is defined as a disorder of

the third stage. About fifteen features have been described as typical for dyspraxia (Johns & La Pointe 1975). At least three such characteristics accuracy strength and tempo of speech all seem to have counter parts in voluntary eye movements

**A Accuracy** The dyspraxic patients seem to be unable to accomplish the se- quence of sounds which form the word. The word is frequently distorted by inaccurate sounds or syllables. Because the sensory verbal systems in these patients are intact they will detect their error and try to correct the error with great effort

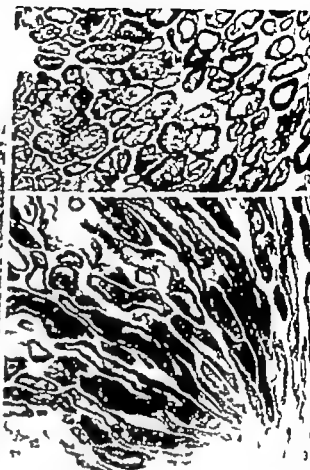
This inaccuracy in speech is reflected in the command eye movements by an inability to execute a saccade of appropriate size. The saccade produced will be inaccurate frequently too small and (as in dyspraxic speech) followed by corrections. It will thus be split up frequently into two or more shorter saccades with or without overshoots.

**B Strength** Strength of dyspraxic speech may be normal with the words uttered with



Fig. 1 Biopsy of the cochlear nerve from a man with 8th nerve neurilemoma. The crushed area in the central parts of the biopsy was brought about by pinching with a pair of forceps.  $\times 115$

Fig. Higher magnification of the site of crushing artifact shown in Fig. 1. On the right the better preserved nerve fibres show myelin coats of normal thickness or occasionally slight thickening of the coat and splitting of the myelin layers. On the left ample myelin material has re-



placed the axons which occasionally are seen as light areas among the myelin material.  $\times 702$

Fig. 3 Longitudinal section of the site of compression. The center of the injured area is at the right lower corner towards which the nerve fibres converge. It appears as if the compressed myelin material had been forced along the lumen of the axis cylinders. No myelin is seen at the site of pinching. Superior vestibular nerve from a patient with Meniere's disease.  $\times 341$

almost totally displaced by thick myelin layer. It often looked as if myelin material had slid away from the site of compression towards the periphery. Axons were present in the area of compression but appeared fragmented or thinner than normal (Fig. 3). Tangential sections showed occlusion of the nerve fibres with myelin material in the zone surrounding the center of compression. Not only thick layers of myelin but also whorls and layers of myelin invaginating into the space between the axon and the myelin coat were seen a little farther from the very site of compression.

Samples taken from the rat also showed

greatly thickened myelin layers (Fig. 4) and at the peripheral sites of damage also myelin whorls and invaginating myelin layers (Fig. 5). The above changes were rarely seen in samples taken after fixation. In places the axons were thicker than normal and in some areas the myelin coats had also ruptured.

Electron microscopy showed denuded and fragmented axons among vesicular debris left by the myelin material in the areas in which the myelin material had been most violently damaged (Fig. 6A). In those areas Schwann cells were no longer seen or ruptured cells or free nuclei were detected. In other places

man daß willkürliche Saccaden in der Rinde des apens ausgelöst werden. Eine Vermehrung des spontanen Nystagmus in den beschriebenen Fällen im Zusammenhang mit einer Entschärfung auf einen Wegfall der kortikalen Inhibition auf die mit den Hirnstämmen.

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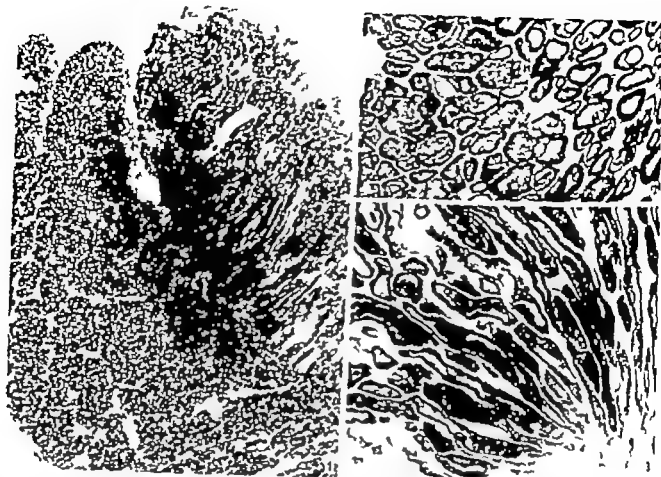


Fig. 1 Biopsy of the cochlear nerve from a man with 8th nerve neurilemoma. The crushed area in the central parts of the biopsy was brought about by pinching with a pair of forceps  $\times 115$ .

Fig. Higher magnification of the site of crushing artifact shown in Fig. 1. On the right, the better preserved nerve fibres show myelin coats of normal thickness or occasionally slight thickening of the coat and splitting of the myelin layers. On the left, amorphous myelin material has re-

placed the axons which occasionally are seen as light areas among the myelin material  $\times 702$ .

Fig. 3 Longitudinal section of the site of compression. The center of the injured area is at the right lower corner towards which the nerve fibres converge. It appears as if the compressed myelin material had been forced along the lumen of the axis cylinders. No myelin is seen at the very site of pinching. Superior vestibular nerve from a patient with Meniere's disease  $\times 541$ .

almost totally displaced by thick myelin layer. It often looked as if myelin material had slid away from the site of compression towards the periphery. Axons were present in the area of compression but appeared fragmented or thinner than normal (Fig. 3). Tangential sections showed occlusion of the nerve fibres with myelin material in the zone surrounding the center of compression. Not only thick layers of myelin but also whorls and layers of myelin invaginating into the space between the axon and the myelin coat were seen a little farther from the very site of compression.

Samples taken from the rat also showed

greatly thickened myelin layers (Fig. 4) and at the peripheral sites of damage also myelin whorls and invaginating myelin layers (Fig. 5). The above changes were rarely seen in samples taken after fixation. In places the axons were thicker than normal and in some areas the myelin coats had also ruptured.

Electron microscopy showed denuded and fragmented axons among vesicular debris left by the myelin material in the areas in which the myelin material had been most violently damaged (Fig. 6A). In those areas Schwann cells were no longer seen or ruptured cells or free nuclei were detected. In other places



Fig 1 Cat nasal mucosa, maxilloturbinal area. Numerous beaded VIP immunoreactive nerve fibres around acini of seromucous glands ( $\times 300$ ).

of both axes and processed for immunohistochemistry.

#### Immunohistochemistry

Specimens were frozen to the temperature of liquid nitrogen in a propane-propylene mixture and freeze-dried. They were then exposed to diethylpyrocarbonate vapour at  $55^{\circ}\text{C}$  for 3 h (Pearse et al 1974) and embedded in paraffin *in vacuo*. Sections  $6\text{ }\mu\text{m}$  in thickness were subjected to the indirect immunofluorescence method (Coons et al 1955) or to the peroxidase-antiperoxidase (PAP) procedure (Sternberger 1974) for the demonstration of VIP. The VIP antiserum (code no 5603) was kindly supplied by Drs J Fahrenkrug & O Schaffalitzky de Muckadell, Department of Clinical Chemistry Bispebjerg Hospital Copenhagen, Denmark. The antiserum has been characterized in detail elsewhere (Fahrenkrug

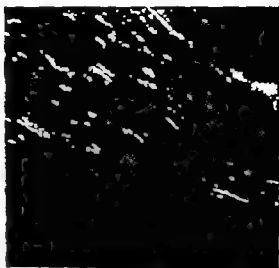


Fig 2 Posterior nasal nerve. Oblique section. Coarse nerve fibres displaying VIP immunoreactivity of varying intensity are abundant in the nerve trunk ( $\times 300$ ).

& Schaffalitzky de Muckadell 1977). The antiserum was used in dilution 1/80 (immunofluorescence) or 1/5600 (PAP procedure) and the site of the antigen-antibody reaction was revealed by fluorescein isothiocyanate labelled sheep antirabbit IgG (SBL, Stockholm, Sweden) diluted 1/20 or by PAP staining. PAP complex (Cappel Labs, Downington Pa, USA) was used in dilution 1/320. Immunofluorescence was observed in a fluorescence microscope with filters selected to give peak excitation at 490 nm. PAP stained sections were examined in a light microscope. Sections incubated with antiserum inactivated by the addition of excess antigen (100  $\mu\text{g}$  of pure porcine VIP per ml diluted antiserum) served as controls.

#### RESULTS

VIP immunoreactive nerves, mostly in the form of delicate beaded terminals were numerous in the nasal mucosa. The nerves were particularly frequent in the maxilloturbinal area. Scattered VIP fibres were also observed in the septum as well as in the ethmoturbinal and nasoturbinal area. Generally the VIP nerves were distributed beneath the surface

with apparent thickening of the myelin coat there was separation of the myelin lamellae (Fig 6B). The site of separation varied but usually occurred at the minor dense line (Fig. 6D). There were areas of swelling around the axoplasm (Fig 6B). Because these areas showed two membranes on the axonal side the swelling was probably in the adaxonal Schwann cell cytoplasm. Such swelling was also seen in samples taken after fixation but it was much more common in biopsies taken before fixation. The separated myelin lamellae were roughly parallel and this pattern was also common in those surgical biopsies which showed compression artifacts. In the latter aggregates of artifactually separated convoluted myelin layers were seen also (Fig 6C). The axon was found to be smaller than usual at the periphery of the fibre. The latter changes however were problematic to interpret. These biopsies also showed changes which most probably were of pathological nature. Because the change was more common in biopsies which showed distinct light microscopic evidence of compression artifact than in biopsies which did not it is probable that the change at least partly was caused by compression.

### DISCUSSION

Artifactual changes corresponding to those we found after deliberate rough manipulation of tissue described above were also noted after the most careful handling of tissue where the nerve tissue was sliced with a razor blade before fixation with glutaraldehyde (Dyck 1975). In both these situations the myelin sheaths appear thicker than normal and show separation of myelin lamellae. Corresponding changes are also shown in the micrographs by Schlote (1971) in his study on traumatic changes of the optic nerve. The myelin sheath may also show abnormal ruptures and increased numbers of myelin whorls inside the sheath.

In certain pathologic conditions such as trigeminal neuralgia and hemifacial spasm

degenerative hypermyelination has been described (Beaver et al 1965, Kumagai 1974). Such changes are very difficult to distinguish from artifacts as shown by our study. In the above conditions other changes also occur (microneuromas which were not seen after mechanical damage) suggesting that the above-described changes are real. Calcium chloride induced neuropathy (Sato et al 1978) may also present nerve fibres with thickened myelin coats. If mechanical damage occurs before fixation these changes may be hidden behind artifacts. On the other hand, numerous artifactual changes in the nerves may suggest degenerative hypermyelination. Under certain conditions interpretation of morphological findings may thus be extremely problematic. Additional methods such as the analysis of teased preparations might then be helpful. The only way of avoiding these artifacts is to avoid mechanical handling but we hope that this description of artifacts may be of some help in cases in which mechanical handling cannot be avoided.

It should also be remembered that fixation may cause artifacts in addition to those caused by handling of unfixed tissues. Beaver et al. (1965a, b) demonstrated fixation artifacts in human trigeminal ganglia after glutaraldehyde fixation. Artifacts due to fixation may also appear in perfusion fixation (Berthold 1968) and it appears that a careful selection of the fixative to be used is necessary in each study. Various alternatives should be tried and the one giving the best preservation should be chosen.

We compared our findings with those described recently by Balentine (1978) in experimental spinal trauma. He found axonal fragmentation 30 min after trauma. We also saw evidence of axonal fragmentation. He observed adaxonal swelling in his biopsies as did we. Likewise he noticed this change in the controls also. This suggests unspecificity of adaxonal swelling. The change is possibly caused by fixation and occurs more often in samples already damaged mechanically. Ve-

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COMPTE RENDU  
DE LA RÉUNION SCIENTIFIQUE DU

COLLEGIUM  
OTO-RHINO-LARYNGOLOGICUM

AMICITIAE SACRUM

BUDAPEST LE 10-13 SEPTEMBRE 1979

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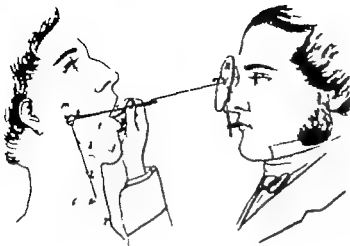


Fig 5 Investigation of larynx with mirror as proposed by Czerniak

prize I feel it was necessary to mention that he came from a Hungarian family

In 1873 Heinrich Neumann was born in Héthárs also in Hungary. He studied in Vienna and the Otologic Section of Francis Josef's

Ambulatorium was at one time under his direction and later he was director of the University ENT Clinic in Vienna.

Finally I must say some words about the Nobel-prize winner Gy. Békésy who died 7



Fig 6 A. Politzer



Fig 7 Gy. Békésy

## EVIDENCE FOR SOUND PERCEPTION WITH THE LABYRINTH

J D Bleeker II P Wit and J H Segenhout

*From the Department of Otorhinolaryngology and the Institute of Audiology  
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(Received February 5 1979)

**Abstract** A report is presented of experiments on guinea pigs and pigeons concerning behavioural and electrophysiological responses of the labyrinth to sound. In the guinea pigs the cochlea was destroyed on both sides by CO<sub>2</sub> laser radiation and fenestration of the lateral semicircular canal was done. In the pigeons the cochlea was removed before fenestration. On account of the results obtained fenestration of the lateral canal in patients with bilateral total cochlear loss can be considered as an alternative to monopolar cochlear implants.

Total cochlear loss on both sides is a serious handicap—even more so when vision is also severely impaired or totally lost. In such cases one is deprived of remote information and is nearly totally dependent on communication through touch and deep sensibility.

Helen Keller (1902) gave us in "The Story of My Life" a stirring account of her feelings and the loneliness to which these patients are doomed. She described how very important every scrap of information is, however meagre, and how other senses, in fact the touch, can be trained to perfection in order to obtain information from outside. This information, however, especially that obtained by touch, is limited to direct contact.

One of the methods used in an attempt to help these patients is direct stimulation of the cochlear nerve with cochlear implants. This treatment has disadvantages we need not discuss here (Bilger 1977, Flottorp 1976, House 1967, Tonndorf 1977, Tonndorf et al 1979). Especially for the multiple electrode implants a rather extensive operation is needed (Chouard 1978).

One might wonder whether the phylogeneti-

cally older sense organ for perception of low frequency vibration in the surroundings, the labyrinth, can obtain sonic information when adequately exposed to sound stimuli. There are arguments to support this assumption.

(a) The fenestration operation, frequently performed 30 years ago in cases of stapes fixation due to otosclerosis, renders the maculae in the vestibule and the crista in the lateral canal accessible to sound vibration in air—especially when, unlike the original fenestration operation, the tympanic cavity and the impedance matching of the middle ear can be left intact.

(b) The Tullio reaction demonstrates that after fenestration a labyrinthine stimulus can be evoked by sound.

(c) Earlier experiments on pigeons have shown that, after the cochlea has been eliminated, a microphonic effect can be obtained by fenestration of, for example, the lateral canal; this effect can be abolished by damaging the ampulla and the maculae in the vestibule (Bleeker 1949, Bleeker & de Vries 1949, de Vries & Bleeker 1949, van Eyck 1955).

(d) Vestibular neurons of squirrel monkeys and fenestrated deaf mice show responses to audio-frequency sound (Young, Fernandez & Goldberg 1977, Mikaelian 1964).

(e) In cats and monkeys a cortical projection of the vestibular system has been demonstrated (Ödkvist et al 1977, Schwarz & Frederickson 1974). So far as we know, this has not been confirmed in man. But certain neurological disorders such as astereognosis are

## SYMPOSIUM

### *Immunological Problems in Oto-Rhino-Laryngology*

Moderator: L. Sutyán

#### *Opening Remarks*

During the last few decades modern immunology has grown into a completely new branch of medicine. Present-day immunological research is concentrated on establishing the site and mechanism of antibody production, on the structure and biological activity of antibodies and on the cellular and molecular basis of immunity. The organism is exposed to the influence of its environment. Various kinds of infections, antigens and allergens invade the mucous membrane and skin. We meet foreign materials of an antigenic or allergenic nature from the first breath and swallow to the last, that is, from birth to death. Therefore throughout our life the recognition of these self and non-self materials is of basic importance. The homeostasis is based on a well organized defence mechanism, which defends the organism whether or not those non-self materials are of an infectious nature.

The organism has mechanical (hair cells), biochemical (enzymes) and immunological defence mechanisms.

The main surfaces on which the organism is in contact with antigens and allergens are the mucous membranes of the respiratory and digestive tracts, a great part of which lie within the field of oto-rhino-laryngology. Therefore we must have a wide horizon to see and judge not only the local reactions and symptoms but also the general ones.

The problem caused by allergic reactions will not be discussed today.

I believe that the basic tenets of immunology are well known to the Members of Collegium and it is therefore superfluous to repeat them on this occasion. On the other hand it

would seem necessary to mention the following few observations or impressions connected with otolaryngology.

(1) I feel that generally from an immunological point of view otolaryngology is on the decline compared with other clinical branches. However one of the important lymphoid organs, the Waldeyer-ring, comes within our field and as was mentioned above the mucous membrane of the respiratory tract represents a very extensive surface on which we meet different kinds of bacteria, viruses etc.

Some of the immunological and inflammatory reactions are observable within the field of oto-rhino-laryngology.

(2) It is necessary to take into consideration that it is not easy to follow the very rapid development of immunology.

Otolaryngologists are much more influenced by surgical techniques, by the good or apparently good effects of antibiotics etc. than by immunological knowledge. In consequence we are not up-to-date in immunology.

(3) A very serious and common mistake is also observable. Classical immunology deals with specific cellular and humoral reactions of the body under the influence of a specific antigen, e.g. tetanus, diphtheria, smallpox etc. and also their theoretical problems but not with the inflammatory reactions caused by bacteria or by the common viruses. The immunological and inflammatory reactions have some similarity but they have far more differences. The inflammatory reactions are also connected with the actions of immunoglobu-



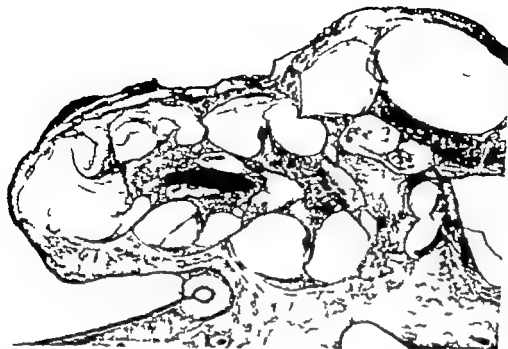


Fig 3 Cochlear destruction after laser radiation. On the surface laser spots. The organ of Corti is destroyed

stimuli. In this way threshold determinations are possible.

In two stages with an interval of 2 weeks the left and the right cochleae were destroyed. The animals were brought under general anaesthesia with a gas mixture of oxygen (1.5 l/min) and fluothane (1.5–2%) a simple and in our experience more reliable method than the often-used anaesthesia with intraperitoneal sodium pentothal (Hoar 1969).<sup>1</sup> On the ventral side, slightly medial to the mandibular angle the bulla was exposed and opened. An equal response curve of  $10 \mu\text{V}$  of the cochlear microphonic was recorded with a single electrode of  $50 \mu\text{m}$  nichrome wire on the outer part of the first turn near the round window. Next the compound action potential for 3000 Hz tone bursts of 3 msec duration at a rate of 10 per second was registered on an oscilloscope screen (Fig 1). Sound pressure was measured with a 1 inch condenser microphone placed close to the animal's ear. The cochlea was then destroyed by  $\text{CO}_2$  laser radiation. Seven shots of 1/10 sec duration were given with an output power of 10–20 Watts depending on the local thickness of the wall of the cochlea. We chose the laser for destruction of the coch-

lea in order to minimize the damage to the labyrinthine structures. The laser radiation caused total destruction of the organ of Corti with extensive damage to the vascular striae in particular (Figs 2, 3, 3a). Two or three weeks after destruction of the second cochlea the animal was fenestrated. Prior to the operation the nystagmogram and the shiver audiogram were checked. The nystagmogram should be normal, the Preyer reflex negative and the shiver audiogram without significant pauses at sound intensities of 100 dB (SPL).

At operation the bullae were opened retroauricularly and the middle ear mucosa was inspected for inflammatory reactions. Next an electrode was placed on the bony wall of the lateral canal near the round window and we checked whether any microphonic or an AP could be registered at maximum sound intensity of about 100 dB (SPL). The noise level of our first set-up made detection below  $5 \mu\text{V}$ .

Laboratory data of the arterial blood after 48 hours narcosis (compared with normal values): pH 7.159 (7.471),  $\text{Pco}_2$  5.69 kPa (3.81),  $\text{Po}_2$  46.85 kPa (10.56), sat 99.7%. We thank L. Prins and J. Bartels for their help in obtaining these data.

# THE FIRST LINE OF DEFENCE

(MODIFIED AFTER DATA FROM T. B. TORRES AND P. BARNETT)

## SURFACE OF THE MUCOUS MEMBRANE

### I PHYSICAL CLEANSING BY THE MUCOCILIARY TRANSPORT SYSTEM

### II UNSPECIFIC PROTECTIVE ACTORS

POSSIBLE ORAL  
ALLOPATHIC  
BIOLOGICALS  
(GLYCOCORTICOLS OF THE MUCOSAL MUCOSA)

TRYPTOPH  
LACTOPERIN  
SECRETORY GLUCOCORTICOLS  
INTERFERON  
COMPLEMENT  
SECRETORY PROTEASE INHIBITORS

### III SPECIFIC ACTORS

IMMUNOLOGICAL  
IMMUNOLOGICAL  
IMMUNOLOGICAL

PROTECTION AGAINST INVASION  
IMMUNOLOGICAL

NEUTRALIZATION OF VIRUSES

NEUTRALIZATION OF TOXINS

TOXIN OF BACTERIAL

OPSONIZATION (PHAGOCYTOSIS)

Fig. The first line of defense (modified after data from T. B. Torres and P. Barnett).

as a response to a virus infection in the infected cells and then prevents further intracellular replication of these microorganisms. Since this agent is available in the affected region only

a few hours after a virus infection, it is also capable of being distributed extracellularly on a relatively large scale and with the secretion. This enables it to keep pace with the viruses which are also replicating and spreading very quickly and to inhibit if not prevent further replication.

The action of interferon is manifold (Fig. 4) although it cannot prevent viruses from penetrating into somatic cells. It can prevent the intracellular replication and spread of very many virus types and even a few other microorganisms. In addition, interferon is a lymphokine and activates the phagocytic activity of the macrophages in the cell-linked immune response. Moreover, this protective substance is quickly available in case of infection whilst it is a well-known fact that specific antibody production does not begin until several days thereafter. The high and polyvalent antiviral effectiveness of interferon has been clearly demonstrated by experimentation in particular in infections of the upper respiratory tract. Unfortunately, interferon is not yet available in sufficient quantities for large-scale clinical use or for more than testing in different virus infections. One of the main problems at present is to find suitable atoxic inducers for interferon synthesis in man.

Another protective factor present in the nasal mucosa and secretion is complement.

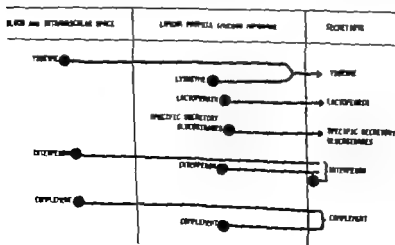


Fig. 3 Unspecifically acting proteins in nasal secretions.

|    | R                   |                    | I                  |                                  |
|----|---------------------|--------------------|--------------------|----------------------------------|
|    | before fenestration | after fenestration | after fenestration | after fenestration               |
| 1  |                     |                    |                    |                                  |
| 2  |                     |                    |                    |                                  |
| 3  |                     |                    |                    |                                  |
| 4  | †                   |                    |                    |                                  |
| 5  |                     |                    |                    |                                  |
| 6  |                     |                    |                    |                                  |
| 7  |                     |                    |                    |                                  |
| 8  | †                   |                    | †                  |                                  |
| 9  |                     |                    |                    | no pre-operative check of shiver |
| 10 |                     |                    |                    |                                  |
| 11 | †                   |                    |                    |                                  |
| 12 | †                   |                    |                    |                                  |
| 13 | pos                 |                    |                    |                                  |
| 14 | †                   |                    |                    |                                  |
| 15 |                     |                    |                    |                                  |
| 16 | res effect          |                    |                    |                                  |
| 17 | †                   |                    |                    |                                  |
| 18 |                     |                    |                    |                                  |
| 19 | †                   |                    |                    |                                  |
| 20 | res effect          |                    |                    |                                  |

Fig 5 Experimental results of 20 guinea pigs 7 animals died before fenestration 4 had to be eliminated The 2 animals with positive results are indicated by arrows

are accessible After opening the recessus scalae tympani the ductus cochlearis can be removed as a whole with a small hook Fenestration is performed by shaving a hole in the exterior part of the lateral canal with a small chisel Care must be taken not to damage the large blood vessel accompanying the canal

Before and after removal of the cochlea microphonic and compound action potentials were recorded with a single electrode (50  $\mu$ m nichrome wire) placed through the hole in the recessus scalae tympani After fenestration recording of the electrophysiological signals was repeated with the electrodes still in the same position as before the fenestration

Schematic arrangements of the apparatus for the electrophysiological measurements are given in Fig 4 (this apparatus became available to us after the completion of the guinea pig measurements) Acoustic stimuli were generated with a Telephonics TDH 39 head phone and lead to the pigeon's ear through a plastic tube Sound pressure calibration was done with a calibrated miniature microphone by connecting this tube to a small cavity in

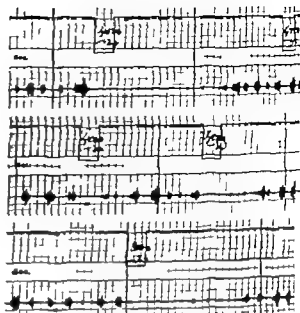


Fig 6 Shiver audiogram of guinea pig no 15 after fenestration Prior to fenestration there was no response Frequency and relative level of the tone burst are indicated in the sound registration line.

stead of the ear of the animal In most of the action potential measurements the acoustic stimuli were presented with alternating phase in order to cancel the microphonic component in the signal through averaging After proper amplification the signal from the electrodes was fed to a modified Princeton Applied Research type TDH # Signal Averager No filtering was applied to the signal during the action potential measurements

The memory content (100 points) of the analogue averager was written out with an XY recorder after smoothing the signal by means of a low pass filter At the end of the measurements some animals were sacrificed for microscopic examination The others are still alive and kept for cochlea extirpation on the other side Further experiments on behavioural responses will possibly be done with these pigeons (Harrison & Furumoto 1971).

## RESULTS

### Guinea pig experiments

The first program has so far been completed in 20 guinea pigs (Fig 5) The extensive pro-

## THE SECOND LINE OF DEFENSE

MODIFIED AFTER DATA FROM T. B. TOMASI AND P. BRANDTZBERG

### MUCOSA PROPRIA

#### UNSPECIFIC FACTORS

- INTERCELLULAR SUBSTANCE AND TISSUE
- MICRO- AND MACROPHAGES
- MAST CELLS
- VESSELS
- AUTONOMIC NERVOUS SYSTEM
- HORMONES
- ( COMPLEMENT INTERFERON
- PROTEASE INHIBITORS ETC )

#### SPECIFIC FACTORS

- SENSITIZED B- AND T LYMPHOCYTES
- EOSINOPHILIC LEUCOCYTES
- IMMUNOGLOBULIN G
- IMMUNOGLOBULIN M
- IMMUNOGLOBULIN E
- (IMMUNOGLOBULIN D)

- NEUTRALIZATION OF VIRUSES
- LYSIS OF BACTERIA,
- NEUTRALIZATION OF TOXINS
- OPSONIZATION,
- FORMATION OF IMMUNE-
- COMPLEXES,

Fig. 10 The second line of defense (modified after data from T. B. Tomasi and P. Brandtzaeg).

of the secretory inhibitor are produced in the mucous membrane itself

(3) It has recently been proven that the high-molecular inter-alpha-trypsin inhibitor in the serum can also be converted in whole or in part into a lower-molecular inhibitor by limited proteolysis. In so doing, it, together with its antiproteolytic activity, thus becomes capable of passing through tissue and is probably passively secreted into the nasal secretion.

It is obvious that the output of the mucosa with these protective proteins is also of outstanding clinical interest. The results of our working group obtained hitherto are already published elsewhere.

Up to now I have mentioned only unspecified protective factors in nasal secretion. Let us now turn to the protective principles dependent on the immune system. Fig. 7 shows first of all that all known immunoglobulins can be present in nasal secretion in principle, although their presence and percentage distribution in the secretion deviates considerably from that in the serum. Rather than give fur-

ther details I will merely summarize the following facts.

IgG represents approximately 80% of all circulating antibodies. It is the principal immunoglobulin in the serum and is distributed as follows: 40% in serum and 60% in extracellular fluids (Alm). IgG occurs in nasal secretion under non-inflammatory conditions in concentrations as can be expected, for example, by transudation from the vascular system. Under inflammatory conditions, however, i.e. in the case of increased vascular permeability, the IgG concentration in the mucosa and nasal secretion is elevated. Under inflammatory conditions, as in the case of chronic infections in the nasal region, the number of IgG-producing plasma cells in the nasal mucosa is greatly increased (Brandtzaeg et al. 1967, 1974). IgG can react with complement and thus activate the chain reaction at C1 or C3.

As far as the mucosa and secretion are concerned, IgG goes into action relatively late after an invasion of microorganisms.

The initial phase for IgM can be shorter

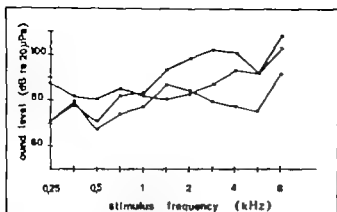


Fig. 9 10  $\mu$ V equal response curves for cochlear microphonics. Individual results for 3 pigeons. Open circles denote data from Ubbens (1955).

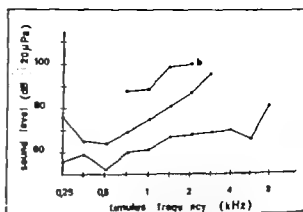


Fig. 10 Average 1  $\mu$ V equal response curves for microphonics (3 pigeons). *a* before cochlea extirpation, *b* after cochlea extirpation, *c* after fenestration. In cases *a* and *c* 10  $\mu$ V equal response curves were measured. The curves given in the figure were derived from these measurements assuming a linear relationship between sound level and microphonic amplitude.

tirpation of the cochlea in none of the animals could an action potential like signal larger than 7  $\mu$ V (average of all the animals 3  $\mu$ V) be detected at maximum stimulus intensity (120 dB (SPL)). One animal died shortly after the fenestration. In the other animals large action potentials could again be measured after fenestration with stimuli lower than 2000 Hz. The shape of these action potentials however is rather complicated (Fig. 12).

Simple-shaped action potentials could be obtained after fenestration with smoothly ris-

ing and decaying single pulses (Fig. 13). The amplitude of the action potential measured after fenestration decreases faster with decreasing stimulus intensity than it does before cochlea extirpation. The maximum amplitude of the action potential measured after fenestration is reached at about 0.5 ms after stimulus onset for the highest stimulus level; this number increases towards about 1 ms for lower

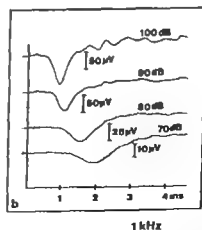
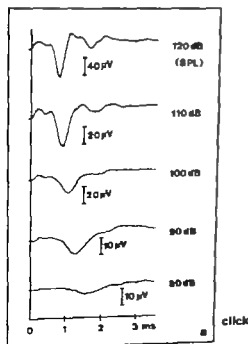


Fig. 11 Compound action potential from a pigeon before cochlear extirpation. In (a) the sound stimulus was obtained by applying one full sine wave (duration 0.1 ms) to the telephone. In (b) the stimulus was a 1000 Hz tone burst (rectangular envelope, duration 4 ms).

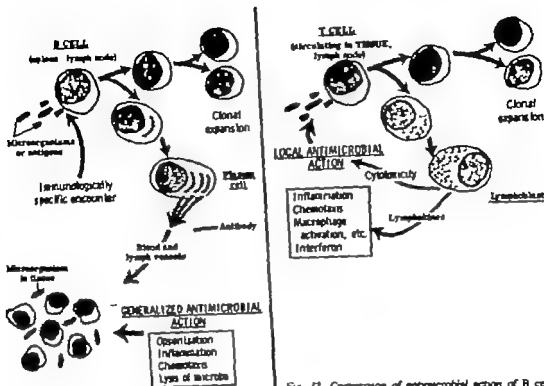


Fig. 12 Comparison of antimicrobial action of B cells and T cells (from C. A. Maza).

orders the immunocomplex in the secretion are resistant to proteolytic activity. Secretory IgA has an antibacterial and in particular antiviral function. It can apparently activate the complement chain by the coaction of lysozyme, leukocytes and complement by way of the C3 shunt, thus triggering bacteriolysis.

Finally, IgE represents only about 0.002% of all serum immunoglobulins. It is produced in the mucosal plasma cells and can also be detected in nasal secretion although frequently only in trace amounts. IgE becomes active as reagent in allergic reactions of the mucosa. Mast cells and basophilic monocytes have receptors for IgE, so that this immune body is capable of very quickly releasing the inflammatory mediators from the mast cells when an appropriate antigen is present.

There is so little significant information concerning IgD that one can pass on to the next point. Once a microorganism has penetrated

the first line of defense represented by the secretory film, it is confronted with a predominantly mechanical barrier (Fig. 9) the formation of the epithelial layer. This barrier however does not appear to be an insurmountable obstacle for microorganisms since on the one hand it is known that the epithelium can be penetrated and destroyed quickly and thoroughly—by viruses—and on the other hand that intravital microscopic observations have shown that even bacteria can pass through the intact epithelial aggregation including the basement membrane (Naumann 1959).

Once the pathogen has passed through the epithelial barrier as well, it is then in the lamina propria of the mucosa and thus in the second line of defense which at the same time constitutes the main line of resistance—tactically speaking.

Depending on the nature and aggressivity of

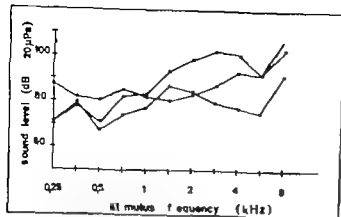


Fig. 9 10  $\mu$ V equal response curves for cochlear microphonic. Individual results for 3 pigeons. Open circles denote data from Ubbens (1955).

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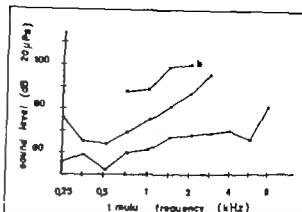


Fig. 10 Average 1  $\mu$ V equal response curves for microphonics (3 pigeons) *a* before cochlea extirpation, *b* after cochlea extirpation before fenestration *c* after fenestration. In cases *a* and *c* 10  $\mu$ V equal response curves were measured. The curves given in the figure were derived from these measurements assuming a linear relationship between sound level and microphonic amplitude.

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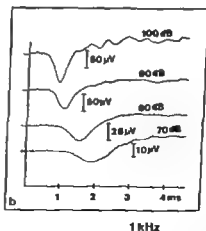
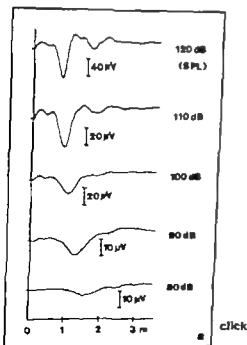


Fig. 11 Compound action potential from a pigeon before cochlear extirpation. In (a) the sound stimulus was obtained by applying one full sine wave (duration 0.1 ms) to the telephone. In (b) the stimulus was a 1000 Hz tone burst (rectangular envelope, duration 4 ms).

## IMMUNOLOGIC ASPECTS OF OTITIS MEDIA

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**Abstract.** The cellular immune response in otitis media with effusion consisted of granulocytic cells in the majority (40-70%) of cases, while the monocytic-lymphocytic cells formed the bulk of cells present in 20%. A considerable number (15-20%) of serous, mucous and mucoid effusions had sparse cellular populations. T-lymphocytes, marked with α-naphthyl-acetate-esterase (ANAE) constituted the majority of lymphocytes and are present in effusion liquids in numbers similar to those in blood. Tests with E-rosettes confirmed the results obtained with ANAE stain. The finding that T-lymphocytes are present in normal numbers may link the mucosal damage to the delayed-type hypersensitivity reaction. Another explanation of mucosal damage may be sought in immune complexes, which have recently been found to be present in many sterile middle ear liquids.

The mechanism of the immunological defence against infectious middle ear diseases has been the subject of intensive studies during the last decade. It has been established that the middle ear mucosa is capable of reacting to bacterial or viral attacks in much the same way as the respiratory mucosa. Secretory otitis media, which has become the most frequent form of middle ear infection in childhood may well serve as a model for histological and immunological studies on the local defence mechanism.

*Immunohistochemical Response*

In 1953 Lahikainen had already shown that middle ear exudates with the exception of liquids deriving from ears in which the infection was caused by *Hemophilus influenzae* had a distinct bacteriostatic effect on the bacteria generally responsible for infection. Lysozyme, the agent thought to be responsible for this effect, is secreted by the middle ear epithelium, and is also liberated from the polymorphonuclear leukocytes and macrophages (Lin et al

1976). Lysozyme as such is a relatively weak agent against bacteria but it can act synergistically with the complement and specific antibodies. In fact, in Lahikainen's studies (1953) heating of the middle ear liquid to 60°C for 30 min to destroy the complement also deprived the middle ear liquid of its bacteriostatic effect. The lysozyme levels in the effusion have been found to be greatly elevated in mucoid secretory otitis media as compared with lysozyme levels in serum (Lin et al 1975, Virtanen & Lahikainen 1979). Similarly the immunoglobulins IgA and IgG show high effusion-serum ratios, while the levels of IgE and IgM have in general been similar to those found in serum (Mogi et al 1974, Lin et al 1976).

Apart from the really rare cases of hypogammaglobulinemia, the majority of chronic secretory otitis media cases show a normal immune response. It must be admitted that there is no appreciable increase in the effused IgM over serum values although this immunoglobulin is known to be particularly responsible for forming antibodies against bacteria. On the other hand, deficiency in IgM production has not been demonstrated. The fact that IgE values are normal proves that in the overwhelming majority of patients the immediate type allergic response is not responsible for secretory otitis media.

The explanation for this prolonged state of infection with low virulence must thus be sought in other mechanisms. In recent years two factors have been brought into focus, viz. possible changes in the T-lymphocyte re-



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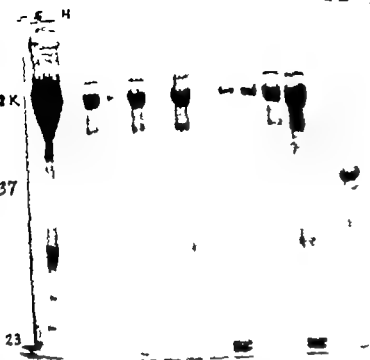


Fig 1 Polyacrylamide gel electrophoresis of 1) middle ear effusions. The references on the left in serum. The two rows on the right represent proteins in the *Escherichia coli* capsule. Molecular weight references are on the left. The protein bands present in the effusions are seen also in the serum.

some 20-25% of specimens showed too small a number of lymphocytes to allow of a reliable estimation of their subpopulations.

For the purpose of determining the T-cell population in the peripheral blood of patients with chronic secretory otitis media, we also used the E-rosetting technique in addition to the ANAE staining. Both methods have in 10 cases given normal figures: the T-cells constituting 60-80% of the cells. E-rosette tests were also made on some cell-rich effusion specimens, generally yielding figures over 50%. However in one effusion specimen the ANAE-positive cells amounted to only 25% and for E-rosettes the result was negative. In peripheral blood, both tests nevertheless gave values as high as 65-75%.

A few tests were also carried out with IgG anti-sheep erythrocyte antibody for demonstration of the Fc receptors. The EA-rosette formation could be seen quite frequently (50-95%) around the macrophages and infrequently (0-15%) with the lymphocytes.

#### Protein Separation with Electrophoresis

41 middle ear aspirates collected from ears having otitis media in an acute or chronic phase were subjected to polyacrylamide gel electrophoresis. Serum and liquids containing proteins of known molecular weight, were used for identification of various protein bands. Liquids containing bacterial capsular antigens with very many sharp protein bands were also employed to ensure a technically faultless separation. An example of such a run is shown in Fig. 1. When a gel with small pores was used much of the mucous material remained at the start. With a gel with larger pores (Fig. 2) the albumin was seen to migrate further towards the anode end but the mucous material nevertheless, remained at the start. The fastest band, present in most middle ear sera, but absent from all control liquids, was at first thought to be specific but on the further testing it proved to be hemoglobin a contaminant in the aspirate. Otherwise there were

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FROM: SEPT C GRAN MATO IN  
 LUCOSE-6 PHOSPHAT DEHYDROGENASE  
 DE FCJ MCT  
 SDN BASK 2 ND ONE  
 OR 3 2 NDROME  
 PL EN DEFIC EN T SYNDROME  
 M A B CHEMO OF LEUCOCYTES  
 2 LEUCOCYTE SYNDROM

12 2

antibody capable of producing a specific bond with it. Under physiological conditions this immune complex will be either filtered out along the RHS channels or—even though it may remain in the organism for some time—will not only be incapable of causing a T-B interaction but even of attaching to the surface of the T cell. There will be no immunoreponse. This phenomenon explains the anti-D prophylaxis and is in some cases responsible for the enhancement reaction.

The sixth possibility is that the antigen with few epitopes produces a complex with an antibody and generates free epitopes. In this event the antibody links with another part of the molecule increasing the epitope count to an extent at which it can bring about a T-B interaction. This will obviously result in humoral antibody production.

The seventh is the very special case in

which the antigen is of cell size. These cells must first be decomposed into protein molecules with free epitopes. Depending on the epitope count, we may have a T or a B immunoreponse.

The eighth possibility is a mechanism which although experimentally verified is far from unequivocal. This is the case in which the affinity energy of the antigen is low and allows only the T to attach to the cell surface. Evidently this specific phase as a process responsible for the evolution of the antibody deficiency syndrome causes the disease either by damaging the macrophages or due to the congenital anomaly of the T or B cells.

Fig. 4 illustrates the development of the immunoparasitus at the cell level, following its evolution from the primordial cell to the immunoreponse. These very complicated differentiation processes are obviously encumbered by innumerable error sources such as a disturbed cell development or the consequences of a lack of regulation. In the latter case we should bear in mind the proportional tasks of the helper and suppressor cells. According to our present knowledge and the experimental nature the two extreme states viz. complete T vs. complete B deficiency provide absolute justification for the existence

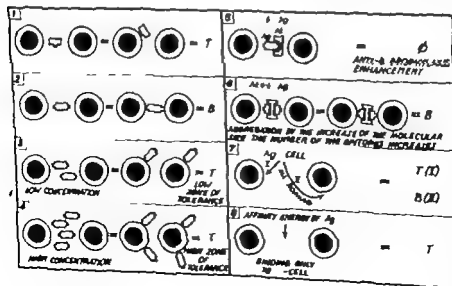


Fig 3

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(KILLER CELLS) ← (ANTI-BLASTIC) (C)  
 (MACROCYT TRANSFORMING ACTOR) ← (ANTI-BLASTIC)  
 (BLASTOGENIC / CTOA)

(MAY TON M)  
 (CLONING INHIBITORY ACTOR)  
 (MAY TON M INHIBITORY CTOA)

(MAY TON M INHIBITORY ACTOR)  
 (MACROCYT AGGREGATING ACTOR)  
 (MACROCYT ACTIVATING ACTOR)  
 (MAY TON M INHIBITORY ACTOR)  
 (MACROCYT AGGREGATING ACTOR)

(MAY TON M)

# GROUP OF IMMUNE-DEFICIENCIES

I M  
 II M  
 III M  
 IV M  
 V M

Fig 7

cases Still more dangerous is the state of humoral immunodeficiency which in the form of congenital diseases may cause the five types of dysglobulinaemia.

The above are the so-called primary antibody deficiency syndromes. Secondary antibody deficiency syndromes also exist.

They may be metabolic disturbances e.g. congenital protein anomalies or the outcome of malignant afflictions of the immunoparatus. They may be caused by the disequilibrium of the chalon systems or the abnormal functioning of the helper and suppressor cells. Catabolic diseases for instance the nephrosis syndrome or enteropathies with loss of protein may give rise to a special secondary antibody deficiency syndrome.

For a successful interaction of the antibodies and cells the distance between two immunoglobulin molecules attached to the membrane should be 300 Å or less thus permitting the complement system to be activated and serineprotease to be generated. Protease disrupts the cell membrane and due to the changed ion flow causes the quasi-explosion of the cell. This proves that the integrity of the humoral immunosystem alone without the activity of the complement system does not ensure adequate protection.

## SECONDARY

HE BULIC  
 ADENOMAS MAY BE  
 AN COBALAM  
 TO BULIC (BULIC)  
 PHORBETICAC NEOPLASIA  
 MAY BULIC MULTIPLE  
 IN WILDER BULIC  
 PLASMOELASTOMA  
 IMMUNOELASTOMA  
 BULIC IN BULIC  
 CARBONIC  
 NEPHROTIC SYNDROME  
 PROTEIN-LOSSING NITROGEN

Fig 8

re differs in any way from that of the individual protein is harmful and to maintain the integrity of the patient its elimination is absolutely indicated. In a cellular immunoreponse a cell-cell interaction takes place which in plain terms means that the organism has little chance of taking up a fight against a large number of tumour cells. This requires an ablative operation with the simultaneous activation of the immunoparatus so as to make it specific—bearing in mind the attempts with a so-called immunotherapy.

Humoral immunoreponse is enhanced by immunoglobulins produced by plasma cells. Different types of immunoglobulins perform different tasks. Some of them are capable of linking the so-called K cells (immunocytes unable to identify) with any tumour cells and destroy them. This is a specific form of the positive interaction of the humoral and cellular systems. There is also a negative interaction when by their very presence the humoral antibodies suspend the cytotoxic effect of the cellular immunosystem for a shorter or longer period of time and bring forth the so-called enhancement reaction. The mere fact that a molecule attaches to molecule, and antigen to antibody is sufficient to eliminate the harmful effect of antigen. However the process is not always so simple partly because immune complexes tend to arise which if not eliminated rapidly may persist in the circulation and lead to so-called immune complex dis-

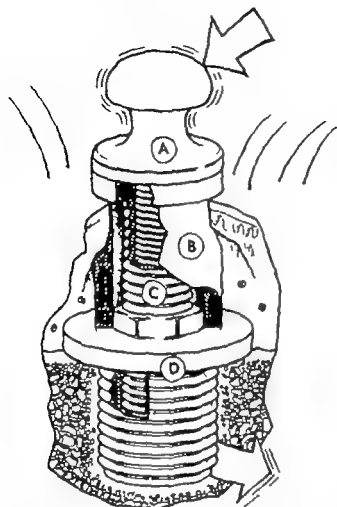


Fig 1 Osseointegrated titanium screw (A) snap fastener (B) titanium abutment, (C) connection screw (D) titanium fixture

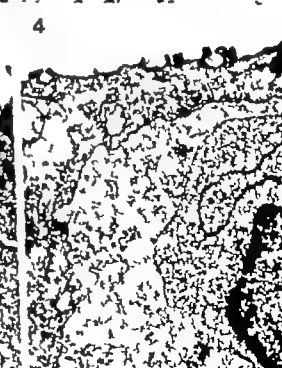
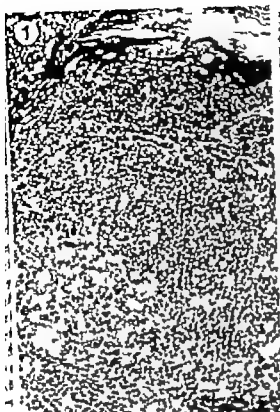
as women who wish to have a more extreme hair style. Thus there is a need to develop a bone conduction hearing aid which minimizes these drawbacks. Our idea has been to use a bone anchored fixture with a skin penetration to attach the hearing aid to. This arrangement could make it possible to construct a small and discrete hearing aid which would eliminate the need of pressure and also eliminate the damping effect of the soft tissue.

#### Osseointegration

The possibility of permanently implanting a foreign material in the body has previously been limited and a permanent reaction free skin penetration has been regarded as essen-

animal experiments (Brånemark & Lundström 1963 Brånemark & Brene 1964 Brånemark et al 1969 Hallén et al 1976) and corresponding clinical experiments (Brånemark et al 1970 Brånemark et al 1975 Brånemark et al 1977 Tjellström et al 1978a Tjellström et al 1978b) shows that titanium has unique properties both in its relation to bone tissue and to soft tissue.

Anchoring of a prosthetical tissue substrate according to the principle of osseointegration means that the surface of the implant connects directly to vital remodelling bone tissue without any intermediary connective tissue sheet. This bio-mechanical connection is obtained by using a combination of careful surgical technique based on biological knowledge about optimization of bone healing and by the use of an inert implant material. Nonalloyed titanium is preferred, the surface of which has special microstructure with an architecture and dimension adjusted to the cellular and interstitial components of the bone tissue. The surface of the implant as well as the bone are protected against all kinds of contamination. An osseointegrated implant can be supplied with extension elements for penetrating skin and mucous membrane which will not be sites for inflammatory processes if the integration is preserved. Experience of these conditions have been collected for more than 15 years. During these years more than 1500 fixtures have been unplanted in the oral cavity to be used for dental rehabilitation. In a pilot study of three patients and a follow up study of 11 more patients, titanium fixtures have been screwed into the bone of the linea temporalis of the temporal bone. All these patients used conventional bone conducting hearing aids previously and were not satisfied with them. After the titanium fixture had integrated with the bone for three to four months a skin penetration was made using titanium abutments held in place by titanium screws which also served as connections for the hearing aids as shown in Fig 1. The longest observation was 36 months and the shortest six





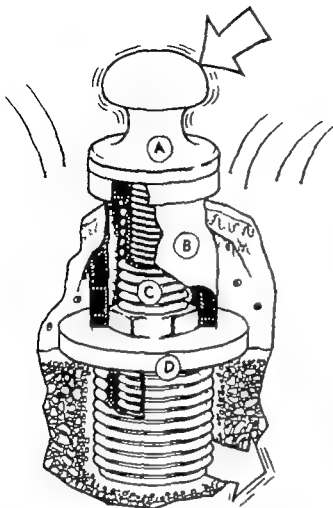


Fig 1 Osseointegrated titanium screw (A) snap fastener (B) titanium abutment (C) connection screw (D) titanium fixture

as women who wish to have a more extreme hair style. Thus there is a need to develop a bone conduction hearing aid which minimizes these drawbacks. Our idea has been to use a bone-anchored fixture with a skin penetration to attach the hearing aid to. This arrangement could make it possible to construct a small and discrete hearing aid which would eliminate the need of pressure and also eliminate the damping effect of the soft tissue.

#### Osseointegration

The possibility of permanently implanting a foreign material in the body has previously been limited and a permanent, reaction free skin penetration has been regarded as essentially impossible to realize. Experience from

animal experiments (Brånemark & Lindström, 1963; Brånemark & Breine, 1964; Brånemark et al, 1969; Hallén et al, 1976) and corresponding clinical experiments (Brånemark et al, 1970; Brånemark et al, 1975; Brånemark et al, 1977; Tjellström et al, 1978a; Tjellström et al, 1978b) shows that titanium has unique properties both in its relation to bone tissue and to soft tissue.

Anchoring of a prosthetical tissue substitute according to the principle of osseointegration means that the surface of the implant connects directly to vital remodelling bone tissue without any intermediary connective tissue sheath. This bio-mechanical connection is obtained by using a combination of careful surgical technique based on biological knowledge about optimization of bone healing and by the use of an inert implant material. Nonalloyed titanium is preferred, the surface of which has a special microstructure with an architecture and dimension adjusted to the cellular and interstitial components of the bone tissue. The surface of the implant as well as the bone site are protected against all kinds of contamination. An osseointegrated implant can be supplied with extension elements for penetrating skin and mucous membrane, which will not be sites for inflammatory processes if the integration is preserved. Experience of these conditions have been collected for more than 10 years. During these years more than 1500 fixtures have been implanted in the oral cavity to be used for dental rehabilitation. In a pilot study of three patients and a follow up study of 11 more patients, titanium fixtures have been screwed into the bone of the linea temporalis of the temporal bone. All these patients used conventional bone conducting hearing aids previously and were not satisfied with them. After the titanium fixture had integrated with the bone for three to four months a skin penetration was made using titanium abutments held in place by titanium screws which also served as connections for the hearing aids as shown in Fig 1. The longest observation time is 26 months and the shortest six

minishes. Due to these changes the possibility of a new infection increases and every infection results in further loss of M cells. The newly formed squamous stratified epithelium is not suitable for antigen uptake but living acteria can penetrate through it to the underlying lymphoid tissue which has fewer Ig-producing cells to defend itself. One can hardly imagine other methods to stop this circular process than tonsillectomy but further work is required to determine whether these changes are reversible or not.

## ACKNOWLEDGEMENT

This work was supported by the Hungarian Ministry of Health (grant no. 5-67-0304-01/13).

## RÉSUMÉ

En cas des inflammation répétées des amygdales linguales l'épithélium recouvert se transforme sur la plupart des surfaces des cryptes en épithélium stratifié et les cellules particulières (cellules M) sont pratiquement absentes. Les cellules M pénètrents renforcent moins de chaînes de transport comme les cellules analogues normaux. Dans les amygdales linguales malades des signes de l'inflammation répétée le nombre de lymphocytes activés est diminué.

## ZUSAMMENFASSUNG

Die Verwänderung der Retikulierung des Kryptenepithel zu einer Antwort des Tonsillengerätes auf entzündliche Reize. Die Zahl der antigenrezeptorischen Epithelzellen (M-Zellen) ist also verringert. Das Zytoplasma der restlichen M-Zellen enthält nur wenige Pinocytosebläschen. Die Zahl der aktivierten Lymphozyten ist in entzündlichen menschlichen Gaumenmandeln auch vermindert.

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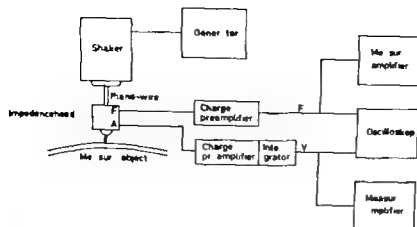


Fig 4 Block diagram of the measurement apparatus

The complex impedance  $Z$  is measured as magnitude (mechanical ohms) and phase (degrees). Calibration was performed with a known mass at 100 Hz. The measurements were carried out with a vibration speed which was neither too low to detect nor too large to be unpleasant for the patients. The accuracy of the electrical measurements is estimated to be  $\pm 5\%$  for the magnitude and  $\pm 2^\circ$  for the phase. Because the system is approximately linear (Flottorp & Solberg 1976) the impedance is insensitive to the applied signal strength.

Measurements have been carried out on eight patients: five women and three men. Their ages varied between 30 and 60 years. In order to simulate the attachment of the hearing aid to the snap fastener, the vibrator and im-

pedance head were suspended from a wheel and balanced with a weight as shown in Fig 5. The patient lay horizontally and had a soft pillow under the head in order to avoid coupling to the supporting structures. To find out what parts of the impedance corresponded to the skull  $Z$  and to the snap fastener  $Z_s$ , separate measurements were made on the snap fastener according to Fig 6. The female element was fastened to a large mass, 63 kg as shown in Fig 6, in order to avoid coupling to the supporting structure.

The measurements were carried out at discrete frequencies between 250 Hz and 8000 Hz. Measurements under 250 Hz are not considered because coupling to the supporting structures can affect the measurement result adversely.

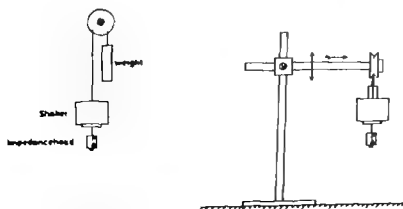


Fig 5 Vibrator and impedance head mounted in a holder which is adjustable both vertically and horizontally

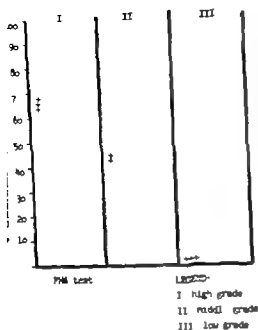


Fig. 2 PHA test and differentiation of the laryngeal cancer

phocytes are stationary and produce through the plasma cells the humoral antibodies—IgG IgA IgM IgD and IgE and IgND (as described by Johanson & Bank in 1967). The latter has been shown to have a cytotoxic effect on tumor cells. It is also supposed that IgM has cytotoxic properties with the help of a complement. It is assumed that some immunoglobulins can enhance tumor growth by coating tumor cells and preventing the killing effect of T lymphocytes and macrophages.

Precursors of T cells migrate from the bone marrow to the thymus where they proliferate and mature into T lymphocytes. These cells circulate in the blood protecting the organism against foreign antigens. They are responsible for graft rejection, the defense against intracellular microorganisms and the recognition and destruction of tumor cells. While the reactive substances in humoral responses are histamines, serotonin, acetylcholine and SRS (on reacting substance), the transfer factor (lymphokines are released in delayed hypersensitivity after the response of the T

lymphocytes. These factors, as for example the chemotactic factor (chemotaxin), the migration inhibition factor (MIF), leukocyte inhibition factor (LIF), macrophage activation factor (MAF), the blastogenic factor (blastogen) and cytotoxin, engage new cells in the reaction. All of this amplifies the initial reaction by T lymphocytes and makes it more effective in the destruction of tumor cells.

Although T and B cells have their own radius of activity, there is still cooperation between them. Among the T cells, there are those that help (helper cells) to promote the lymphocyte reaction, but also those that can suppress (suppressor cells) the immune reaction. Likewise, alpha globulins have a suppressive effect on T cells and it is considered that the multiplication often prevents immunosuppression.

Our trials conducted in 1959 on laryngeal cancer revealed that immunoglobulins were elevated in the majority of cases and that their increase paralleled the growth of the tumor. The opposite was found to be true for the circulating lymphocytes, which were few in advanced stages of the disease.

Riesco described a positive correlation between the number of peripheral lymphocytes and the incidence of tumor cure and a negative correlation between the number of peripheral neutrophils and the incidence of cures. T cells can be detected quantitatively by rosette formations and qualitatively by the blastogenic test or the DNCB test.

When correlating PHA percentages and the absolute number of T lymphocytes in laryngeal, hypopharyngeal and skin cancer, values were 50% or more below normal in laryngeal and hypopharyngeal cancer and normal in skin cancer (Fig. 1). There was also a positive correlation between the maturity of the tumor and PHA values. Out of the 12 cases of laryngeal cancer in Grade I, the PHA findings were normal in 7 patients and distinctly low in all 6 of the cases in Grade III.

In 53 patients who underwent surgery for laryngeal cancer between 1972 and 1977,

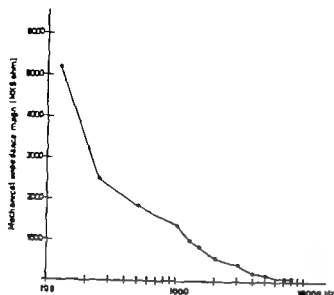


Fig 10 Mechanical impedance of the snap fastener

vidual variations and poorer precision in registering the results. The results of measurements on the snap fastener are shown in Fig 10. A scattering caused by the tightness of the snap fastener (tight, medium loose) was also noted. That scattering is about  $\pm 15\%$  for frequencies below 800 Hz and very small for higher frequencies.

## DISCUSSION

The scattering in the measurement results is mainly explained by insufficient precision in the instruments, errors in reading the instruments and varying tightness in the snap fastener used. Variations in different skulls regarding elasticity and weight could also influence the scatter (Kylén 1977).

The measurement results are described in an electrical analog model in which the block

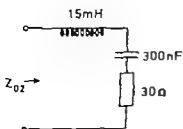


Fig 11 Electrical analog model of the snap fastener

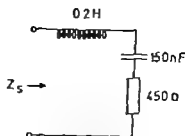


Fig 12 Electrical analog model of the skull impedance in frequencies over 2 kHz.

elements in Figs 2 and 3 are replaced by  $R$  and  $L$  combinations. The results of the measurements on the snap fastener give a model according to Fig 11. The resonant frequency is then 7.5 kHz. This means that the inductance can be neglected for frequencies below 7.5 kHz. A comparison between the impedance of the snap fastener  $Z_m$  and that of the head shows that the impedance of the snap fastener determines the total impedance for frequencies over 2 to 3.5 kHz. This means that at higher frequencies the impedance of the skull is considerably greater than that of the snap fastener.

For frequencies below 2 to 3.5 kHz one finds that the total impedance  $Z_s$  has a resonant frequency at about 800 Hz. According to Kirikae (1958) the skull has its lowest resonant frequency between 1.7 and 1.9 kHz. Kirikae's measurements were carried out on an unattached skull. The resonant frequency found in our measurements at about 800 Hz can be due to the attachment to the neck and spinal cord. An electrical analog of the impedance of the skull  $Z_s$  is shown in Fig. 12. This model is not valid for frequencies over 2

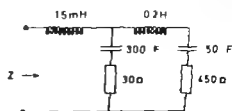


Fig 13 Electrical analog model of the combined system snap fastener and skull

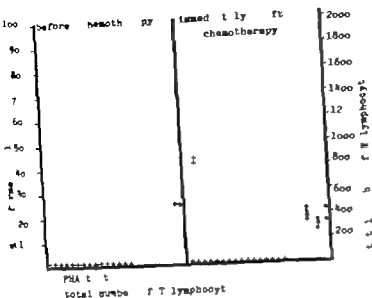


Fig 5 PHA test and total number of T-lymphocytes following chemotherapy

(1976) reported similar results. Therefore in addition to evaluating the patient's cellular immunity more attention must be focused on the local lymphocytic reaction around the tumor.

### THERAPY AND THE IMMUNOLOGICAL RESPONSE

We wanted to investigate how the immunologic responses of the organism with

laryngeal cancer change during various kinds of treatment (surgery, irradiation and chemotherapy) and thus followed the values of the PHA test and the absolute number of lymphocytes. In 21 patients with laryngeal cancer (10 with partial laryngectomy, 3 with partial laryngectomy and RND, 5 with total laryngectomy and 3 with total laryngectomy and RND) we checked these values before and 4-7 days after surgery and could not con-

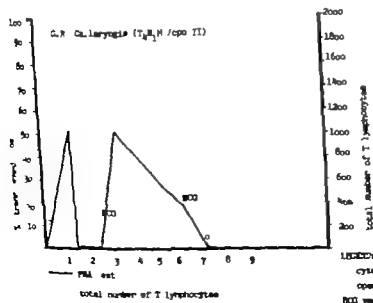


Fig 6 PHA test and total number of T-lymphocytes in patient following surgery, irradiation and chemotherapy

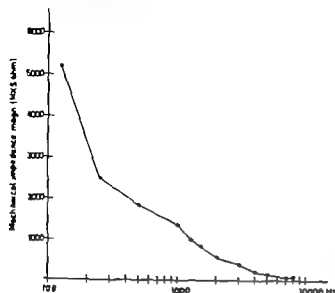


Fig 10 Mechanical impedance of the snap fastener

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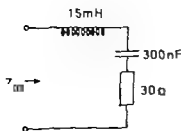


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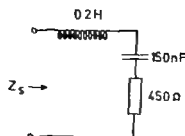


Fig 12 Electrical analog model of the skull impedance for frequencies over 1 kHz.

elements in Figs 2 and 3 are replaced by  $R$ ,  $C$  and  $L$  combinations. The results of the measurements on the snap fastener give a model according to Fig 11. The resonant frequency is then 7.5 kHz. This means that the inductance can be neglected for frequencies below 7.5 kHz. A comparison between the impedance of the snap fastener  $Z_s$  and that of the head shows that the impedance of the snap fastener determines the total impedance for frequencies over 2 to 3.5 kHz. This means that at higher frequencies the impedance of the skull is considerably greater than that of the snap fastener.

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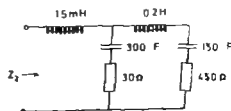


Fig 13 Electrical analog model of the combined system snap fastener and skull

## CONCLUDING REMARKS

L. Surján

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We have listened to some very interesting papers dealing with the oto-laryngological aspects of immunology and of inflammatory reactions. I am sure that there will be an increase in interest in immunology inspired by these interesting and informative lectures.

I should like to add a few comments.

(1) In oto-laryngology one important problem occurring in the case of acute inflammation. It seems to be forgotten that *ubi pus ibi evacua*. "Where there is pus, it should be drained." This old Greek saying remains valid in our days too. It can be observed that myringotomy has been avoided and the treatment of acute otitis media is based solely on antibiotics.

(2) It is not enough to give antibiotics against the infections—it is also necessary to take into consideration the reactions of the body and to help the defence mechanism. I believe that if the therapeutic treatment of acute inflammations of middle ear as adopted nowadays

were better than the earlier one, we would not observe so many cases of chronic otitis media as occurred before the antibiotic era. And it is also a fact that a definite increase is observable in the numbers of adhesive processes and tympanosclerosis which originate from an earlier inflammation.

(3) It seems that there is controversy regarding the timing of antibiotic application. The optimal time for commencing this treatment is not determined and the long-term results have not been investigated.

(4) The real effect of antibiotics given preventively is also questionable. We do not know what kind of body reactions occur in these cases!

(5) Finally I should like to suggest that we oto-laryngologists ought to think not only about the technical problems and about antibiotic treatment but also to bear in mind the defensive immunological and inflammatory reactions of the body.



he found that a large amount of sound energy is absorbed by the skin (Békésy 1960). The same findings have been noted by Kylén (1977) in his amplitude analysis.

### Psychoacoustical aspects

Some initial psychoacoustical measurements have been performed but the results are still preliminary. The patients who have received bone-anchored hearing aids emphasize that the loudness increases greatly and that the volume control on their apparatus must be turned down to a minimum in order to get an agreeable sound level. They further emphasize that the frequency range is significantly increased in comparison with conventional bone transmission. These two factors mean that the quality of the sound reaching the cochlea can be improved. Much less power will be required to drive a hearing aid using an osseointegrated titanium fixture. At present the hearing aid fitting is being optimized for this new situation.

### ZUSAMMENFASSUNG

Einige auf Hörgeräte angewiesene Patienten können nicht einen Apparat, der den Schall durch den äußeren Gehörgang vermittelt, benutzen, sondern müssen ein knochenleitendes Hörgerät anwenden. Das Gerät ist mit Hilfe eines Stahlbügels gegen die Haut des Processus mastoideus gedrückt und der Schall ist durch die Weichteile und Knochengewebe zu der Schnecke vermittelt. Der Druck, der das Hörgerät verursacht, ist für den Patienten unangenehm. Die dämpfende Wirkung der Weichteile verringert die übermittelte Schallqualität. Die Möglichkeit, fremdes Material reaktionslos zu implantieren, um eine permanente Hautpenetration durchzuführen, zu können, haben es ermöglicht, ein knochenverankertes Hörgerät herzustellen. Vierzehn Patienten sind mit solchen Apparaten versehen. Wegen des neuen Verfahrens muß ein neues Gerät hergestellt werden. Die Impedanz der knochenverankerten Titanschraube/Schädel wurde untersucht. Die Resistenz und Reaktanz der mechanischen Impedanz wurden gemessen. Die Einwirkung der dämpfenden Weichteile wurde analysiert. Der Unterschied zwischen der Impedanz des Schädels und die der Weichteile + Schädel betrug abhängig von der Frequenz, etwa 10–25 dB.

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Fig 3 Electron micrograph of serous gland showing lumen (L), lined with few microvilli (M), intercellular canaliculi (AC), finger-like cytoplasmic processes (Pr), many desmosomes (D) and terminal bars (T). The cells

contain fewer free ribosomes (R), granular endoplasmic reticulum (GER), small mitochondria (M) Golgi apparatus (G) and nuclei (N) 9 625

scattered between the granules. No intact zymogen granules were noted in the lumina of the acini. There was diminished activity of the cell organelles in the form of fewer free ribosomes, smaller mitochondria and Golgi apparatus and fewer dilated cisternae of the granular endoplasmic reticulum.

In mucous cells (Fig. 4) the mucus droplets

were abundant, almost completely filling the supranuclear cytoplasm, with marked propensity to fuse laterally with neighbouring droplets, producing relatively large, irregular, membrane-bound aggregates of mucus. They appeared paler and of nearly the same electron density. Only a few scattered organelles were evident in the interstices between the closely

he found that a large amount of sound energy is absorbed by the skin (Békésy 1960). The same findings have been noted by Kylén (1977) in his amplitude analysis.

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Some initial psychoacoustical measurements have been performed but the results are still preliminary. The patients who have received bone anchored hearing aids emphasize that the loudness increases greatly and that the volume control on their apparatus must be turned down to a minimum in order to get an agreeable sound level. They further emphasize that the frequency range is significantly increased in comparison with conventional bone transmission. These two factors mean that the quality of the sound reaching the cochlea can be improved. Much less power will be required to drive a hearing aid using an osseointegrated titanium fixture. At present the hearing aid fitting is being optimized for this new situation.

### ZUSAMMENFASSUNG

Einige auf Hörgeräte angewiesene Patienten können nicht einen Apparat der den Schall durch den äußeren Gehörgang vermittelt benutzen, sondern müssen ein knochenleitendes Hörgerät anwenden. Das Gerät ist mit Hilfe eines Stahlbügels gegen die Haut des Processus mastoideus gedrückt und der Schall ist durch die Weichteile und Knorpelgewebe zu der Schnecke vermittelt. Der Druck der das Hörgerät verursacht ist für den Patienten unangenehm. Die dämpfende Wirkung der Weichteile verringert die übermittelte Schallqualität. Die Möglichkeit fremdes Material reaktionslos zu implantieren um eine permanente Hautpenetration durchführen zu können, haben es ermöglicht ein knochenverankertes Hörgerät herzustellen. Vierzehn Patienten sind mit solchen Apparaten versehen. Wegen des neuen Verfahrens muß ein neues Gerät hergestellt werden. Die Impedanz der knochenverankerten Titanschraube/Schädel wurde untersucht. Die Resistenz und Reaktanz der mechanischen Impedanz wurden gemessen. Die Einwirkung der dämpfenden Weichteile wurde analysiert. Der Unterschied zwischen der Impedanz des Schädels und der der Weichteile + Schädel betrug abhängig von der Frequenz etwa 10–25 dB.

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Fig 7 Electron micrograph of an arteriole showing lumen (L) endothelial cells (E) endothelial basement membrane

(ELm), muscle cells (MC) and elastic fibres (EL) in the tunica media 5775

periglandular capillaries and they narrowed down as they approached the lumen. This diminished secretory activity is consequent to lesser exposure to infection and lesser need for defensive mechanism.

The preponderance of the goblet cells in the epithelium is a balancing mechanism to compensate for the diminished number and activity of the mucous acini, both of which secrete the same mucigen. This provides the mucous

blanket covering the epithelial surface which is necessary to maintain ciliary function.

The diminution in the number of cilia, the delicacy and loosening of the epithelium, the diminished activity of the seromucinous glands with diminished production of bactericidal lysozyme indicate a lower resistance of the sinus mucosa to infection and easier penetration for micro-organisms.

There is a diminished exchange between tis-

he found that a large amount of sound energy is absorbed by the skin (Békésy 1960). The same findings have been noted by Kylén (1977) in his amplitude analysis.

### Psychoacoustical aspects

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as 1975) shown that cell-to-cell junctions in an epithelium normally constitute a tight barrier for molecules of such a size.

The second mechanism foresaw the passage through the epithelial cell by a process of endocytosis. It was later shown by South et al (1966) that this is the probable mechanism of transport for IgA that secretory IgA differs from serum IgA in which a protein is supplied to them during the transport through the epithelial cell and this is called "transport piece". The function of this piece is together with the J component to facilitate the transport and allow for the successive polymerization of IgA (Tomasi, 1972).

The purpose of this work is to provide direct evidence of this second mechanism and to evidence the role of cellular organelles involved in this secretory process so as to suggest a description of the integrated sequence of cellular events connected to it by means of electron immunomicroscopy of human nasal mucosa.

## MATERIALS AND METHODS

(a) *Use of immunoenzymatic techniques in electron microscopy* They are employed to localize specifically a cellular product or component by the relative antibody conjugated with an enzyme as a marker. The reaction product of this enzyme with relevant endogenous or exogenous substrate produces electron-dense formations which are therefore clearly visible at the electron microscope (Williams, 1977a).

To identify the localisation of IgA in the nasal mucosa we have used complexes formed by anti-human IgA antibodies or (as a control) anti-human IgG antibodies (Dako USA) conjugated with Horse Radish Peroxidase (HRP, Sigma) using glutaraldehyde as a cross-linker according to the two-step method devised by Avrameas and described by Williams (1977a).

(b) *Procedures for the immunoenzymatic technique and for E. M.* Biopsies of normal human nasal mucosa taken from the middle

turbinate have been cut into thin slices and immersed into 2% glutaraldehyde in phosphate buffer 0.1 M pH 7.3 with  $\text{CaCl}_2$  for about 10 min and then transferred into a solution containing 1 mg/ml of IgG-anti-IgA + HRP conjugate or IgG-anti-IgG + HRP (control) or only buffer (control for endogenous peroxidase) and incubated for 20–30 min with continuous gentle agitation.

The specimens were washed and incubated for one hour in a 1% Diaminobenzidine (DAB) solution at room temperature according to the technique suggested by Graham & Karnovsky (1966) and described by Williams (1977a). This step was necessary to carry out the reaction between the enzyme (HRP) and the substrate (DAB). Because the reaction product is highly osmophilic, the following 2 hours post-fixation on 1.33% osmium tetroxide gave the osmium-black products easily identifiable at the E. M. in unstained sections.

The post-fixed specimens were washed in the same buffer, dehydrated in ethanol and embedded in Epon-812. Ultrathin sections were observed without any staining with a Philips 300 electron microscope.

(c) *Quantitative evaluation* The sporadic presence of endogenous peroxidase and thereby of the reaction products has made a semi-quantitative evaluation of the enzymatic reaction products necessary. We have followed the techniques and the recommendations by Weibel (1968) described by Williams (1977b).

## RESULTS AND CONCLUSIONS

The osmium-black reaction product has been found specifically abundant in all epithelial cells of nasal mucosa. In particular the Golgi apparatus showed a highly electrondense material both in the cisternae of dictyosomes and related vesicles (Fig. 1).

The large secretory granules (Fig. 2) had osmium-black product localized at the periphery near the limiting membrane. The small granules and primary or secondary lyso-

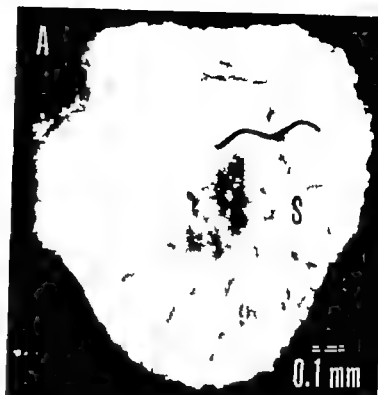


Fig 1 Normal otoconial membranes A the macula utriculi B macula sacculi right ear The curved line in A indicates the shape of an imaginary cross section of the snowdrift line (S) which consists of a single ridge (S) in the saccule. OsO

(1973a) has postulated that the so-called dark cells in the wall of the utricle are capable of removing calcium from otoconia which have become attached to them

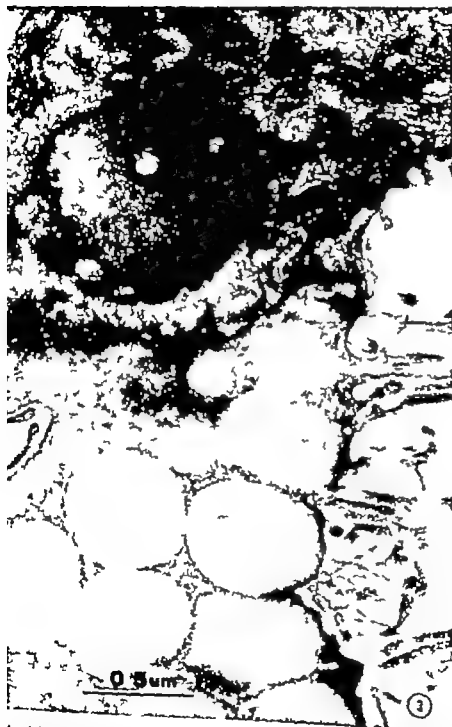
During the last decade there has been a growing interest in the pathology of otoconia. Abnormalities of otoconia are usually associated with a marked reduction of the crystalline layer of the otoconial membrane and an unusual size and shape of the remaining crystals. The most common form of otoconial pathology in man is the marked loss of otoconia occurring with aging. The loss is much more severe in the saccule than in the utricle to the extent that the macula sacculi may completely lack otoconia in older patients (Johnsson & Hawkins 1972a, b; Ross et al 1976).

Johnsson & Hawkins (1979) have described otoconial defects in man where the integrity of the vestibular epithelium or membranous wall was severely disturbed. Under the influence of head trauma or ear disease otoconia may become dislodged from the utricular macula and enter the posterior ampulla pro-

voking a disorder which Schuknecht (1974) has termed cupulolithiasis.

Complete absence of otoconia from all four vestibular maculae in a 6-week-old infant was recently reported by Wright, Hubbard & Graham (1979b). This anomaly, which apparently was congenital in origin, was strikingly similar to that present in certain genetically defective animals such as the pallid mouse in which otoconia fail to develop (Lyon 1951). Otoconial defects are now known to occur in at least five different mutant rodents and in one species of partridge (Lum & Erway 1974). Also abnormal otoconia have been observed in both the saccule and utricle of the deaf Dalmatian dog (Johnsson et al 1973) which like the mutant mice suffers from a genetically determined pigmentation defect.

Acid solutions and other decalcification agents can obviously alter or dissolve the otoconia which consist of calcite, i.e. calcium carbonate crystallized in trigonal form. The otoconial membranes are also very sensitive to mechanical trauma. The membranes be-



*Fig. 3* Detail of the above picture. The smallest secretion granules with a homogeneous content do not contain the reaction product. On the contrary the electron-black

reaction product is well visible at the periphery of the large granule with dense and homogeneous content. Unstained section 60000





Fig. 2. Abnormal-appearing pair of otoconial membranes from the right ear of two untreated normal pigmented guinea pigs. U: macula utriculi. S: macula sacculi. A mild (top) and a more severe (bottom) degree of reduction of otoconia in the crystalline layer of the otoconial mem-

brane. The apparent loss of otoconia is more diffuse and severe in the sacculus (lower right) while it is clearly concentrated along the striae in the utricle. Numerous otoconia are scattered in the sacculus. OsO<sub>4</sub>.

concentrations of alcohol (35% 50% 70%). The largest number of specimens was dissected 2–4 days after fixation but particular care was taken to dissect several specimens on the day of sacrifice in order to confirm the presence of abnormal findings prior to any lengthy storage in alcohol. The examination of the otoconia was done primarily with the dissection microscope. A few otoconial membranes were studied as surface preparations in phase-contrast and dark field illumination. The technique of microdissection used in this

study is described in detail elsewhere (Håkms & Johnsson 1976).

#### MATERIAL

Thirty two colored guinea pigs which had received no drugs served as the normal material. Furthermore two series of 75 and 53 albino guinea pigs were examined. Several of these animals had been treated with streptomycin or neomycin. An additional series of 8 normal albino animals were also studied. T

## RESUMÉ

Le rôle des anticorps sécrétés IgA dans la défense muqueuse et antibactérienne des hautes voies aériennes a été amplement démontré et la détermination des IgA dans la sécrétion nasale est devenue, à notre avis, de la plus grande importance dans l'étude clinique rhinologique. Le mécanisme par lequel l'IgA arrive à la sécrétion nasale à travers la muqueuse est encore objet de discussion entre les deux hypothèses de Tomasi (libre filtration à travers l'interface épithélio-connectif ou transport unimoléculaire avec sécrétion active) en jusqu'ici de démonstration véritable.

En employant une IgG anti IgA humaine purifiée injectée par de la peroxydase de horseradish, nous avons pu d'une façon semi-quantitative sur des sections histologiques fines, le comportement de l'IgA au niveau de muqueuse nasale humaine.

On peut démontrer que l'IgA élaborée et sécrétée dans la muqueuse connectif par les cellules plasmocytiques spécifiques, serait incorporée par endocytose par les cellules épithéliales, ou elle est visible surtout dans les corps de Golgi et dans les grandes granulations de sécrétion. Les données du transport endocytotique démontrent le rôle de la pinocytose car le rôle est étroitement joué par les microfilaments actine et par les microtubules, avec utilisation de l'ATP. Il serait intéressant d'investiguer en employant soit des agents physiques (tels que le froid) qui empêchent l'utilisation de l'ATP soit des agents chimiques (tels que la tetracycline<sup>3</sup> et la colchicine) qui dépolymérisent respectivement les microfilaments et les microtubules, si l'on peut obtenir des preuves ultérieures en faveur du mécanisme de transport actif de l'IgA suggéré par les résultats de nos études présentes.

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## DISCUSSION

Surjan J. to Crifo: You have presented nice description of IgA transport and modern sensitive method was applied. The result is similar to that of Brannstrom. The presence of IgA in the Golgi of epithelial cells is, however surprising. In this respect please let me know how was your anti-IgA purified and how is the specificity proved?

Crifo (Reply)

Thanks to Dr Surjan Jr for his comment. The sensitivity of the electron-immunofluorescence is more valuable than the immunofluorescent method. Such technique permits study at the level of endocellular organelles in this way is possible the reconstruction of the dynamic events occurring during or after given phenomenon. I would be grateful for any advice giving me notice or previous documentation of IgA-active transport at the level of the mucosa membrane. Naturally all people retain today this mechanism more probable but we have not found in the literature any documentation on it, similar to that which have reported.



Fig. 2 Abnormal appearing pair of otoconial membranes from the right ear of two untreated normal pigmented guinea pigs U macula utriculi S macula sacculi A mild (top) and a more severe (bottom) degree of reduction of otoconia in the crystalline layer of the otoconial mem-

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Table I Comparison of two laboratory technicians' readings of 1127 cytotoxic tests

|                                      | Test results recorded by Lab Technician A |     |    |     |      | Total |
|--------------------------------------|---|-----|----|-----|------|-------|
|                                      | -   | +   | ++ | +++ | ++++ |       |
| Results recorded by Lab Technician B | 814                                       | 54  | 18 | 1   | 0    | 887   |
| +                                    | 54  | 29  | 20 | 7   | 0    | 110   |
| ++                                   | 26  | 27  | 23 | 4   | 0    | 79    |
| +++                                  | 6   | 7   | 11 | 18  | 2    | 44    |
| ++++                                 | 0   | 0   | 1  | 4   | 2    | 7     |
| Total                                | 900                                       | 117 | 72 | 34  | 4    | 1127  |

are added to the heated serum before incubation with food antigens.

(c) To block  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions: one drop of 1% EDTA (ethylenediamine-tetraacetic acid) distilled water was added to 17 drops of serum and the buffy coat leukocytes. This solution was used in place of the distilled water normally used in cytotoxic tests.

(d) To inhibit the possible histamine release from the leukocytes: one drop of 1:5:10 and 0.5% DSCG (disodium cromoglycate) solution was added to the leukocyte-food antigen preparation.

(e) To block possible H<sub>1</sub>-receptors on the cells: one drop of antihistamine solution Phenergan<sup>®</sup> 5 mg/ml Tavegil<sup>®</sup> 1 mg/ml was added to the cell suspension.

(f) The effect of steroids on the cytotoxic reaction was studied by substituting cortisone compound solution (Betnesol<sup>®</sup> 1:25000) or hydrocortisone 1:1000 (1:100) for the drop of distilled water in the cytotoxic tests.

4. Histamine chloride and histamine acid phosphate were used in concentrations of 1:1000, 1:100, 1:10 and 1:5 in order to study the effect of histamine on leukocyte pseudopod formation. The solutions were made from the powder in sterile distilled water and substituted for the distilled water used in the cytotoxic test.

## RESULTS

1. Identical results were recorded in 78% of 1127 tests read double-blind by two laboratory technicians. A slight divergence in regard to the severity degree of the reactions occurred in 16% and a greater deviation in 6%. The statistical error of the method was 1/46 (Table I).

2. In the series of tests repeated on 3 consecutive days 81% of the results recorded for the first day agreed with those noted for the second day, 80% of the test readings for the first and second days showed agreement and 82% of the tests, second-day and third-day results were concordant. The statistical errors were 0.41, 0.43 and 0.38 respectively.

3. In 47 of 48 tests (77%) a positive leukocyte reaction was transferred with the reactive serum to cells from non-reactive subjects. By

Table II The effect of heating (56°C, 2 h) on the cytotoxic reaction

|                         | Number of positive reactions | Per cent |
|-------------------------|------------------------------|----------|
| Original cytotoxic test | 17                           | 100      |
| Heated serum            | 4                            | 46       |
| Washed cells            | 11                           | 1        |

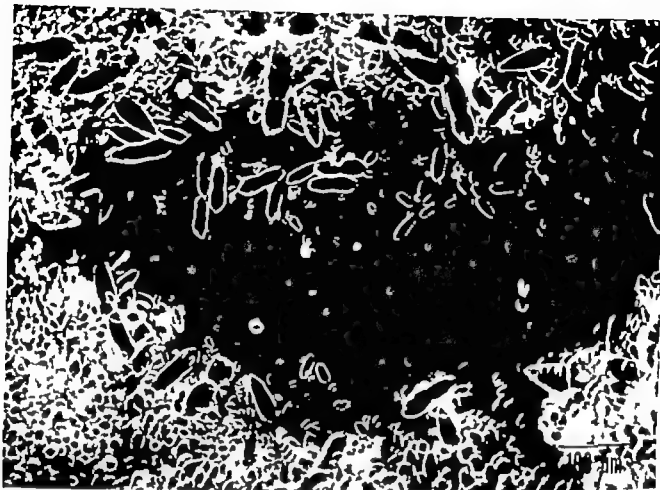


Fig. 4 Detail of a defect similar to that shown in Fig. 3. Several large dumb-bell shaped otoconia, up to 90  $\mu\text{m}$  in length are seen.

conial layer extended over the whole surface of the macular neuro-epithelium and 2) animals in which the otoconia covered only a part of the neuro-epithelium. The otoconial membranes of Group 1 were rated as 'normal' those of Group 2 as 'abnormal' because the latter deviated so markedly from all previous descriptions of the macular organs in normal mammals (Figs 1-5).

#### *Normal otoconial membranes*

There is little new information in the present study to be added to the many descriptions of normal otoconial membranes in guinea pigs (Fig. 1). The line of the snowdrift on the saccular otoconial membrane resembles that seen in man. On the macula utriculi on the other hand a single relatively sharp ridge was not

present as in human specimens (Johnsson & Hawkins 1967; Wright & Hubbard 1978; Wright, Hubbard & Clark 1979a). Instead, two low ridges were seen running in parallel to form the usual 'U' shaped line. Between these there was a shallow groove. Lateral and posterior to this double line of snowdrift the surface was more rippled than elsewhere, often forming a pattern of small ridges which tended to radiate toward the margins of the membrane more or less perpendicular to the snowdrift line (Fig. 1). When the opposite side of the membrane which faces the neuro-epithelium was examined a more darkly stained curved line was seen in the gelatinous part of the membrane under the snowdrift. In agreement with Lindeman's (1969) earlier observation we found that the membrane alone

actions. The results are thus somewhat contradictory and it seems that the cytotoxicity in the leukocytes must also be caused by one factor(s) other than complement action. Whether for instance the lymphocytes play a role via liberation of lymphokines remains an open question.

Factors possibly connected with histamine release and its blocking were tested by adding CG and antihistamine to the leukocyte-antigen preparation. The protection provided by DSCG may probably be attributable to the stabilizing effect which this drug exerts on the cell membrane. Antihistamines were found to inhibit expected reactions in 93% of tests. This can hardly be due to the H<sub>1</sub>-receptors competing successfully on the cellular level but it is more likely that antihistamines have acted as cell membrane stabilizers. Similarly, cortisone when present in a sufficiently high concentration around the cell seemed to have a stabilizing effect on the cell membrane. However, these *in vitro* results do not necessarily imply that the same effects would be produced *in vivo*.

To sum up, the cytotoxic leukocyte reaction appears to be a multifaceted process which is effected by factors as yet unknown. The reproducibility of the test is fairly good provided that the technicians are properly trained. The definite significance of the cytotoxic test in the diagnosis of food allergy is yet to be elucidated. The test usually gives negative results in so-called fixed food allergy and its importance may well lie in the diagnosis of chronic, cyclic food allergy. However, not all positive reactions in a test can be taken to indicate clinically manifest food allergy and provocation tests and/or elimination diets are necessary in order to determine whether the foods indicated by the test can be held responsible for symptoms.

## RÉSUMÉ

Les réactions ont été faites dans 49 spécimens du sang et les réactions établies par deux techniques à la

manière du double-blind. Les résultats identiques ont été trouvés dans 78% des cas avec une erreur statistique de 0.46. Dans une groupe de 32 malades, le même test cytotoxique a été répété en 3 jours consécutifs. Des résultats différents ont été trouvés dans 18-20% des tests avec une erreur statistique de 0.38 et 0.43. Spécimens du sérum (75 sujets) avec une réaction cytotoxique leucocytaire après un contact avec des antigènes alimentaires ont été étudiés et les observations suivantes faites. Dans 42 des 48 tests, la réactivité a été transférée avec un sérum positif dans les leucocytes normaux. L'échauffement à +36°C pendant 2 heures et le lavage des cellules ont éliminé la réactivité dans 44 cas de 52. Le traitement des cellules avec EDTA dans 36 spécimens complètement bloqué la réaction cytotoxique. DSCG a bloqué la réaction dans 45%, les antihistaminiques dans 93% et la cortisone dans 20% des tests.

## ZUSAMMENFASSUNG

Mit Blutproben von 49 Patienten wurden im Doppel-blindversuch zytotoxische Tests durchgeführt und von zwei Laboranten geprüft. In 78% der Tests waren die Resultate identisch und der statistische Fehler der Methode war 0.46. In einer Reihe von 32 Patienten ist der zytotoxische Test an 3 aufeinanderfolgenden Tagen wiederholt worden. Die Auswertung ergab verschiedene Resultate in 18-20% und statistische Fehler zwischen 0.38 und 0.43. An Serumproben von 75 Personen mit zytotoxischen Leukocytenreaktionen auf Nahrungsmittelantigenen wurde im Anschluß an zytotoxische Tests verschiedene *in vitro*-Experimente angestellt. Die folgenden Beobachtungen wurden gemacht: 1. 4 von 38 Tests wurde die Reaktivität mit dem Serum zu inaktiven Zellen überführt. Erhitzung der Serumproben während Stunden in einem Wasserbad von 36°C und Waschen der Zellen eliminierten die Reaktivität in 44 von 52 Proben. 2. 36 Testen erreichte Behandlung mit EDTA ausnahmslos eine totale Blockierung der zytotoxischen Reaktion. DSCG blockierte die Reaktion in 45%, Antihistamin in 93% und Kortison in 20% der Tests.

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In the abnormal specimens the reduction of saccular otoconia was quite variable. Characteristic findings are shown in Fig. 2. Compared with the utricle the apparent loss of saccular otoconia was invariably more extensive. The more severe the changes were in the saccule the more pronounced was the defect in the utricle. In both saccule and utricle numerous otoconia were found scattered around the macula and adherent to the membranous wall, although the latter phenomenon was mostly confined to the utricle. We observed no otoconia in the ductus reuniens or in the cochlea. Moreover, there seemed to be no tendency for the otoconia to fall into the posterior ampulla.

During dissection the otoconia became dislodged more easily from the defective membranes than from the normal specimens, apparently because the otoconia were less adherent to the gelatinous membrane in the abnormal specimens. The otoconia varied greatly in size. When the reduction of saccular otoconia was severe the crystals often appeared translucent rather than milky white and some of them had a roundish shape.

On the abnormal utricular maculae there was a marked reduction of otoconia in the area where the snowdrift was present in normal specimens. This gave rise to a deep groove in the otoconial layer which left the gelatinous portion of the membrane uncovered and transparent. The darkly stained neuroepithelium was visible through the bare parts of the membrane, creating the appearance of a black curved strip above the stria. The posterior half of this strip was always wider than the anterior half.

As illustrated in Figs. 3 and 4, single otoconia which were visible in the area of the defect were usually much larger than elsewhere on the membrane and they often had a peculiar dumb-bell shape, much like the otoconia described by Lim et al. (1978) in the tilted-head mutant mouse.

In surface preparations the reinforcement of the gelatinous layer of the otoconial membrane above the stria could clearly be seen (Fig. 5). In general the appearance of utricular membrane was similar to that in normal guinea pigs, although the saccular membrane in the guinea pig lacked the striking and complicated arrangement of fibrils seen in man (Johnsson & Hawkins, 1967).

In the animals with an incomplete set of otoconia the number of crystals attached to the surface of the membranous wall of the utricle was usually greater than in normal animals. In both the normal and abnormal utricle the otoconia on the wall had the appearance of a thin layer of powder snow. The distribution of such crystals was not random. Circumscribed white areas were seen covering areas of the wall which stained darker than the surroundings. In most guinea pigs a transverse sharp border could be discerned separating a translucent light zone of the wall above the anterior third of the macula from a darker posterior region. A sharp line of demarcation was usually present, created by a layer of otoconia covering the darker area (Fig. 6). In the samples of utricular wall studied by light microscopy it could be seen that the otoconia which were adherent to the wall were often clustered within the boundaries of single cells. This finding seems to be similar to what has been demonstrated by Lim (1973a) in a scanning electron micrograph. When a large number of otoconia were present the distribution was more even and the characteristic pattern in Fig. 6 could not be seen. These otoconia varied in size, but most of them were small, approximately  $1\text{ }\mu\text{m}$  in length. They were highly refractile and they did not appear to be in the process of degeneration.

In 2 animals from the series of 8 albino guinea pigs studied a single giant irregularly shaped otoconium or otolith was found on each macula (Fig. 5). Only one ear was available from each of these animals; it is therefore not known whether the anomaly was bilateral.

Table 1 Statistical description of rhinomanometric and radiographic measures

| N  | M     | S.D. | Min. | Max. |
|----|-------|------|------|------|
| 23 | 2.99  | 1.58 | 1.1  | 7.3  |
| 4  | 4.83  | 2.91 | 0.0  | 9.0  |
| 4  | 8.38  | 3.57 | 0.5  | 15.0 |
| 4  | 11.48 | 5.25 | 1.0  | 19.5 |
| 4  | 4.44  | 2.64 | 0.0  | 10.0 |
| 4  | 18.75 | 3.51 | 1.5  | 29.0 |
| 4  | 30.58 | 4.77 | 22.0 | 39.5 |

L: Nasal respiratory resistance in cm H<sub>2</sub>O/lsec

P: T: T: Radiographic measures (Fig. 1) in mm

1. The shortest distance from the upper face of the palatine velum to the adenoid bed

2. Depth of the soft tissue

3. The soft tissue shadow on a line from pterygomaxillary point to the midpoint of line joining basion and the center of sella tursica (Linder Aronson, 1970)

4. The soft tissue shadow on a line from pterygomaxillary point to basion (Linder Aronson 1970)

On the postero-anterior radiographs the 1st of the airway was measured as the post distance between the lateral choanal orders

#### Rhinomanometry

The NRR was measured with a commercially available rhinomanometer the Mercury Electronics Nasal Resistance Meter NR1. The instrument was modified to permit anterior as well as posterior recordings and an infra digital display was added to permit recording in the natural respiratory rhythm. A method for individual lining of the nose mask was developed for tight fitting. A biological feedback procedure based on an oscilloscope display of the flow-pressure diagram gave information to the patient on the proper method of breathing. In this way all children down to the age of 5 years could participate in the examination. For a detailed description of the methodology see Solow & Greve (1979)

1980) Posterior as well as anterior rhinomanometry was performed. In the present study only the posterior recordings were used.

The measurements were calculated as the mean of four series each comprising four recordings at a constant flow threshold of 0.1 l/sec for the anterior recordings and 0.2 l/sec for the posterior recordings. The method error within a session was found to be 0.2 cm H<sub>2</sub>O/lsec.

Before the recording Neosynephrine® 0.5% was administered as nose drops in order to eliminate variations in the NRR due to congestion of the nasal mucosal membrane.

*Surgical assessment* of the size of the adenoids. The amount of removed tissue was given a semiquantitative evaluation recorded as - + ++ +++.

## RESULTS

The presurgical figures from rhinomanometry and radiography are given in Table I. The most narrow passage in the nasopharyngeal airway was found at P and P. All measurements of passage showed wide inter-individual variations. The depth of tissue was always most pronounced at T. The mean at T<sub>1</sub> was 18.75 mm which exceeds significantly the limit of 15 mm set by Johannesson (1968).

The radiographic measures of the nasopharyngeal airway P<sub>1</sub>-P<sub>2</sub> showed highly significant intercorrelations ( $r=0.83-0.95$ ,  $p<0.001$ ) and the measures P<sub>1</sub>-P<sub>2</sub> showed correlation of about -0.5 ( $p<0.05$ ) with NRR (Table II Fig. 2). The amount of adenoid tis-

Table II Correlations between radiographic and rhinomanometric measures of the nasopharyngeal airway

| Radiography    | Rhinomanometry<br>NRR |
|----------------|-----------------------|
| P <sub>1</sub> | -0.52                 |
| P <sub>2</sub> | 0.47*                 |
| P <sub>3</sub> | 0.46                  |
| P <sub>4</sub> | -0.38                 |

\*  $p < 0.05$



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## Table III Correlations between presurgical symptoms

|                | RUAI | Snoring | Rhinolalia | Mouthbreathing |
|----------------|------|---------|------------|----------------|
| AI             |      | .19     | .55        | .00            |
| nas            | .19  | -       | .42        | .48            |
| rhinolalia     | .55  | .42     |            | .40            |
| mouthbreathing | .00  | .48     | .40        |                |

n=85

pharyngeal measures of the nasopharyngeal airway and NRR was found for the dimension P (Robert & Whitehouse 1978) i.e. the short distance from the most anterior part of the nasal mass to the posterior wall of the sphenoid sinus which is usually found in the upper part of the nasopharynx. P also showed higher correlations with Snoring than with P<sub>2</sub>. Moreover Fig. 7 shows that in the subjects with the highest presurgical values of LR, the predominant indication for adenoidectomy was Snoring. These observations suggest that a high location of the nasal tissue leads to increased NRR and snoring.

Among the symptoms considered, the best relation was found between RUAI and rhinolalia. Recurrent Upper Airway Infection is often considered an indication for adenoidectomy. Judged by radiography however children with RUAI did not present reduced size of the passage in the nasopharynx. Their main disease thus may have been located in the nose, a finding which suggests that RUAI alone is not indication for adenoidectomy.

The correlation analysis further demon-

strated an association between Snoring and Mouthbreathing. This relates to the finding that the highest correlation between the radiographic dimensions and Snoring was found for the dimension P. The mechanism behind these relationships could be that elevation of the soft palate in Mouthbreathing reduces the distance P<sub>4</sub> between the velum and the adenoid tissue and facilitates the oscillatory movement of the soft palate causing Snoring.

The radiographic measures of the depth of the adenoid tissue (T<sub>1</sub> and T<sub>2</sub>) and the size of the airway (especially P and P<sub>2</sub>) showed rather similar correlations with impaired nasal breathing (Snoring and Mouthbreathing). This indicates that both types of measurement are of value in determination of the clinical indication for adenoidectomy.

The relation of tubal dysfunction to nasopharyngeal airway is outside the scope of this study. According to Mawson (1968) and Rynnel-Dagöö (1978) recurrent ear inflammation and secretory otitis media are not *per se* indication for adenoidectomy.

The present study suggests that adenoidectomy should be performed only in children with obstruction of the nasopharyngeal airway.

Table IV Correlations between presurgical symptoms and measures of the nasopharyngeal airway

| Symptoms       | Nasopharyngeal airway |       |                |                |                |
|----------------|-----------------------|-------|----------------|----------------|----------------|
|                | NRR                   | P     | P <sub>2</sub> | P <sub>4</sub> | P <sub>5</sub> |
| RUAI           | .38                   | .03   | .13            | .1             | .08            |
| snoring        | .33                   | -.42* | .28            | .36            | .45            |
| rhinolalia     | .26                   | .03   | .10            | .06            | -.00           |
| mouthbreathing | .30                   | -.25  | -.17           | -.30           | -.28           |

n=85

Table V Correlations between presurgical symptoms and measures of adenoid tissue

| Symptoms       | Adenoid tissue |                |
|----------------|----------------|----------------|
|                | T <sub>1</sub> | T <sub>2</sub> |
| RUAI           | -.08           | -.07           |
| Snoring        | .40            | .41            |
| Rhinolalia     | .00            | .05            |
| Mouthbreathing | .31            | .38            |

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## CHOLESTEROL GRANULOMA

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**Abstract** The idiopathic haemotympanum is in reality a permanent secretory otitis media. Histologically it demonstrates chronic granular otitis media associated with cholesterol granulomas. This, however, is not a very specific finding and may be frequently found in other types of the "moderated middle ear syndrome" such as ears with central perforations or indeed with cholesteroloma. In these granulomas, iron deposits are found, as well as the iron-chelating agent—lactoferrin, which is known to be part of the defence mechanism of all mucous glands and is specifically secreted by the exocrine glands and it is not surprising to find much of it in the middle ear in situations where mucus is being abundantly synthesized—as the case may be in any otitis media. There is no real clinical or histological evidence so far that neither the iron deposits nor the cholesterol crystals of these granulomas originate from previous haemorrhage.

"Idiopathic haemotympanum" is a condition where the tympanic membrane is tinted blue—and is therefore also called "The Blue Ear drum". On exploration these middle ears are found to contain granulomas in which cholesterol crystals as well as iron deposits can be demonstrated. The purpose of this study was to see whether lactoferrin, an exocrine gland-secreted iron-chelating protein, is associated with these granulomas and the iron therein.

## METHODS

The tissue to be examined was quick frozen on dry ice immediately after removal and stored at  $-80^{\circ}\text{C}$ . Later it was cut with a cryocut into  $4\text{ }\mu\text{m}$  thick sections at  $-70^{\circ}\text{C}$ . Mucus was examined after it was smeared on glass slides. The sections and smears were dried and subsequently incubated with anti-lactoferrin antiserum (DAKO Copenhagen, Denmark) diluted 1:8 for 30 min at room temperature, rinsed in saline phosphate buffer, pH 7.2 (PBS) for 20 min, incubated with a fluorescein-conju-

gated anti IgG (DAKO) diluted 1:20 for 70 min at room temperature, rinsed again in PBS for 70 min, mounted in buffered glycerin, covered with a coverglass and observed under ultraviolet light with a Zeiss Standard Universal microscope with epifluorescent condensor. The magnification was  $\times 400$ . Pictures were taken with a semi-automatic camera. The presence of lactoferrin was demonstrated by an apple-green fluorescence. Negative preparations showed no fluorescence.

## MATERIAL

141 specimens from 51 patients were examined, grouped as follows:

A. 10 samples of mucus, retrieved from secretory otitis media ears.

B. 3 biopsies from the promontorium of ears taken during tympanometry (for stapedectomy) from 3 patients.

C. 15 biopsies from cholesterol granulomas from 5 patients.

D. 17 biopsies from the mucosa and granulation tissue from 4 ears with dry chronic otitis media, which came for tympanoplasty.

E. 45 biopsies from the mucosa granulation tissue from 9 patients having wet thick mucosa in simple chronic otitis media who came for tympanoplasty.

F. 20 biopsies from the mucosa, granulation tissue and matrix, from 9 ears with cholesteatoma, which came for surgery.

G. 30 biopsies from salivary glands taken during various salivary gland surgical operations from 10 patients.

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cherry red or pink) and coat color. Animals with defective otoconial membranes were found in all categories of eye and coat color. In our small sample it happened that the incidence was highest (4 out of 9) in the pink-eyed animals with light coats (e.g. yellow tan light grey) and lowest (4 out of 17) in animals with dark eyes and dark coats. A total of 10 of the 32 colored guinea pigs had otoconial defects.

In the series of 75 albino guinea pigs used in the  $^{45}\text{Ca}$  uptake study, some of which were treated with neomycin and streptomycin, 14 animals had abnormal otoconial membranes. The overall incidence of animals rated as abnormal in the combined sample of 107 guinea pigs was 22.4%. In a subsequent series of 53 animals which included both untreated as well as streptomycin and neomycin treated animals, 17 animals with abnormal otoconia were found.

## DISCUSSION

It could be argued that what we have described as an abnormality in the vestibular system of seemingly normal animals simply represented a normal variation of the mass number, distribution and structure of the otoconia. As mentioned above, however, the otoconial membranes rated as abnormal differed so markedly in appearance from previous descriptions and from what we have observed in a variety of animals including monkeys, dogs, cats and several species of rodents that they evidently must represent some form of anomaly or pathology. We have found similar defects of the otoconial layer in the utricle of the rat and gerbil but in no instance was the deviation from normal appearance as marked as in the guinea pig, nor was the incidence nearly as high.

It is noteworthy that the pathological otoconial membranes occurred in colored as well as in albino guinea pigs. One can only speculate about the possible cause for the finding. We do not know whether the abnormality was

congenital or was acquired after birth, the question of whether the finding represents agenesis, dysgenesis or degeneration of the otoconia therefore remains open. Considering the relatively young age of the animals, the most likely explanation for the presence of the abnormal otoconial membranes is a congenital hereditary condition, perhaps resulting from long term inbreeding.

Because of the sparsity of otoconia in abnormal specimens, numerous crystals were studied in detail, but a comparison with normal samples was difficult because the otoconia were not as well displayed in the dense crystalline mass of normal membranes, particularly in the deeper layers. Therefore, it could not be unequivocally established that the large, unusually shaped otoconia which we observed (Figs 3-4) were actually abnormal in guinea pigs. Only the specimens shown in Fig. 5 were analyzed by powder X-ray diffraction. Judging from the shape of the otoconia, we found other abnormal membranes, however, most of the crystals probably consisted of calcite.

Although systematic behavioral testing was not carried out in the present study, the animals with otoconial defects (including those having single giant crystals on each of the maculae) exhibited no obvious postural or behavioral abnormalities. With regard to this observation, it might be noted that Lim et al. (1978) have shown that animals with genetically determined otoconial defects often display normal air-righting reflexes and swimming ability, even though the total area of sensory epithelium actually covered by otoconia may be much less than normal. These workers postulate that even a single large crystal attached to the gelatinous otoconial membrane may provide a uniform stimulus for a large area of the neuro-epithelium. In addition, the absence of overt behavioral signs might to some extent reflect the intervention of central nervous compensatory mechanisms brought into play in the presence of congenital defects of the peripheral receptor organs.

The function of any of the accessory oto-

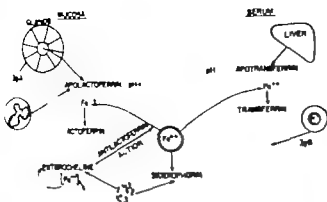


Fig 4 Diagram showing how various factors, belonging to bacteria and host, compete among themselves for available iron.

et al. 1966a 1966b) of which the middle ear by virtue of its respiratory epithelial lining is also a part (Sadé 1966). Neutrophils also produce lactoferrin (Schafer 1951). Since its discovery in 1939 by Sørensen (1939) interest in lactoferrin has grown and numerous studies have been made of it (Biserie et al. 1963; Blanc & Isliker 1961; Johansson 1960; Maasson et al. 1964 1966a 1966b; Montreuil & Muller, 1960; Sadé 1966; Schafer 1951). Lactoferrin is a glycoprotein to which iron ions bind reversibly with a molecular weight of 80 000 daltons (Maasson et al. 1966b; Blanc & Isliker 1963; Schade et al. 1949). When it is secreted by the mucosa in an iron-free form it is termed apolactoferrin. This system present in exocrine excretion, is very similar to the humeral iron-chelating system—the transferrin-apotransferrin system, which is a plasma protein synthesized in the liver. In analogy to the IgG and IgA system we also have here a double anti-bacterial system—the one functioning in the circulation (transferrin and IgG) the other on mucosal surface (lactoferrin and IgA).

The antibacterial action of the iron-chelating agents is related to the dependence of bacteria and fungi on the presence of iron (Arnold et al. 1977; Hansen et al. 1975; Kirkpatrick et al. 1971; Reiter et al. 1975). Microorganisms are devoid of iron deposits and are therefore in constant need of iron supplies: they develop and multiply according to the amount of iron available. In mammals however iron is usual-

ly not present in a free form but is protein-bound. It can be retrieved by bacteria by means of their own iron-chelating agent called siderophore which can transfer iron ions from host to parasite. Apparently lactoferrin is called in to counter this activity. The host-parasite competition for iron is complicated by an anti-lactoferrin agent produced by bacteria, termed enterochelin (Rosenberg & Young 1974) (Fig. 4). Bullen et al. (1971 1972) also supplied evidence to suggest that lactoferrin can act synergistically with specific antibodies and provide a more powerful antimicrobial system than either component alone. The production of enterochelin is increased by bacteria in the absence of iron and the capacity to produce it is consequently a factor in bacterial virulence (Kochan et al. 1977). Lactoferrin activity is highest at a pH below 7, transferrin at pH values above 7. This prompted one of us (A. H.) to suggest that some of the bacteriostatic effect of lowering the pH as performed for example in chronic middle ear infections by using boric or acetic acid could be related to this.

To sum up the presence of iron deposits in a secretory mucosa, as found by us in blue ears in middle ear mucus or any other chronic and inflamed middle ear mucosa could therefore be explained as secondary to the chelating effect of lactoferrin. The finding of lactoferrin in these instances is not really surprising, as lactoferrin is secreted by most mucosae—of which the middle ear is only one.

## ZUSAMMENFASSUNG

Die Otolithenmembran vieler albino- und normalen pigmentierten Meerschweinchen wurden mit Lichtmikroskopie untersucht. Es stellte sich durch das Mikrozieren heraus, daß die kristalline Schicht der Otolithenmembran unvollständig war, denn man fand große Teile der gelatinösen Fläche unbedeckt von Kristallen. Diese Defekte wurden an beiden Seiten gefunden, sie waren mehr oder weniger symmetrisch und ausnahmslos stärker im Sacculus vertreten als im Utriculus. In zwei Meerschweinchen wurden einzelne große Anhäufungen der Kristalle gefunden, an der Macula sacculi sowie an der Macula utriculi. Keines der Tiere zeigte Anzeichen von anormalem Benehmen und anormaler Haltung, und man fand keinen auf der Hand liegenden Grund für die Otolithendefekte. Die Anwesenheit einer vererbten angeborenen Veranlagung ist naheliegend. Das häufige Vorkommen der Otolithendefekte in Meerschweinchen macht diese Tiere ungeeignet für die meisten Untersuchungen der versuchsweise eingeführten Pathologie. Darum sollten im Hinblick auf die jetzt vorliegenden Untersuchungen vorherige Studien der Otolithen-Pathologie neu überprüft werden.

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ways as a chelate agent or as an SH group donor. In the first case we have to assume that copper and other metals are necessary to taste sense; in the second that thiol groups interfere with taste acuity.

The copper serum concentration in patients and animals treated with some drugs rich in thiols and exhibiting ageusia is normal yet copper administration restores the taste acuity. Although both drugs have an identical chemical structure it is difficult to think in terms of a chelating mechanism in Homo-cysteine because it has been supposed that this drug does not deplete copper (Henkin et al 1969). Hence we wonder how it is that copper and other metals can restore taste sensitivity. Perhaps they bind the excessive SH concentration for another mechanism.

The preneural phase of taste could hypothetically be influenced by metals and/or thiol concentrations that would regulate the effectivity of the contact between the tastants and the receptors. Beidler (1954, 1970) assumed that the gustatory stimuli interact with specific sectors of the plasma membrane of the receptor cell. He characterized these sectors as receptor sites. According to Vinnikov (1974) the most recent biochemical investigations have confirmed that the interaction of a taste stimulus with receptor sites is based on physical processes of the absorption type and not on enzymatic processes; this is also Beidler's point of view.

The sensation of taste can be regarded as sensitivity to chemical substances in solution (Jenkins 1978) that have to make contact with the receptor site. The hypothesized interaction between metals and thiols must be one of making that contact easier or more difficult.

In any case this first regulation would act upon the four taste qualities, as has been confirmed by clinical and experimental experiences affecting the acuity but not the quality.

Murray & Murray (1970) consider the taste pit in which a little known dense substance exists to be the region of primary reaction in taste appreciation. The dense substance is

considered by Vinnikov (1974) to be an analogue of ion-exchange resins which are capable of refining gustatory substances in such a way that their gustatory stimulating chemical source can interact with the receptor surface, i.e. with the plasma membrane of the microvilli present in the sensory cells. Consequently this substance must play a protective role simultaneously.

Some of us (Ciges et al 1978) studied the dynamics of pit region and observing that the taste buds desquamation process regulate in some way the size of the pores and the amount of dense substance which covers the ends of all but the most extended sensory hairs.

We attach great importance to this substance in the primary events of the taste process because it covers the receptor sites and we assume that the action of metals and thiols must be effected upon the dense substance. It might be an equivalent to the mucus covering the olfactory epithelium both substances being chemical elements corresponding to the nature of the stimulus. On the other hand physical senses such as hearing or labyrinth have physical devices such as tectorial membrane, cupuli and otoconium which play an important functional role.

In order to contribute to a better knowledge of the different biochemical processes—and particularly the interactions between metals and thiols—that probably take place during the first events of taste we have carried out a double investigation in two different fields.

First, Determination of copper concentration in ageusic animals by Acetyl-homocysteine and D-Penicillamine at different levels not only in serum as is usual but also in sensory tongue mucosa, saliva, salivary glands, liver, heart and muscle. Knowing that the liver plays a central role in the maintenance of copper homeostasis and its storage along with muscles we thought that it would be worthwhile investigating these organs.

In tissues and body fluids there are true copper metalloproteins which are not in the same class (Sass-Kortsak & Bearn 1978) as



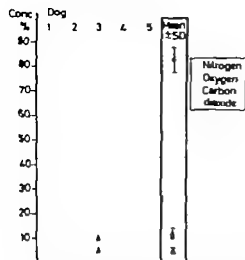


Fig 1 Composition of middle ear gas of air ventilated dogs (individual values mean and standard deviation)

(Sadé et al 1976) Under those conditions we were able to obtain usually 40–60  $\mu$ l of MEG. The gas samples were examined on the day of collection.

The MEG composition was determined by a Packard 836U Gaschromatograph with a thermal conductivity detector using a combination of Porapak and 5A Molecular Sieve column system as previously described (Ostfeld et al 1979).

## RESULTS AND DISCUSSION

The determinations were carried out on 5 dogs. The composition of the MEG as obtained in the individual dogs is shown in Fig 1.

The following mean content ( $\pm$ SD) of gases in ME of air ventilated dogs was found: N<sub>2</sub> 83.2%  $\pm$  2.5, O<sub>2</sub> 12.1%  $\pm$  2.2 and CO<sub>2</sub> 4.7%  $\pm$  0.7. The ranges were from 80 to 86% for N<sub>2</sub> from 10 to 15% for O<sub>2</sub> and from 3.7 to 5.5% for CO<sub>2</sub>.

The present study of the composition of the MEG in air ventilated dogs has been undertaken as a preliminary step in our investigation on changes occurring under various conditions of general anesthesia. The dog has been chosen as an experimental animal because its ME volume is similar to that of humans and because it enables the use of an anesthetic

technique identical with that used in clinical surgical work. Hopefully the observations obtained in dogs may be valid and useful also for understanding the physiological processes related to MEG in man.

During the sampling of MEG special attention has been given to the prevention of inadvertent leakage of air as cautioned by Drettner (1975). This was achieved by covering the TM with a 4–5 mm seal of saline (Sadé et al 1976).

Matsumura (1955) analysed the MEG composition of cats and men by Krogh's gasometric microanalytic method in which the volumetric percentages of the gases were determined following absorption of CO<sub>2</sub> by KOH and O<sub>2</sub> by an alkalinized solution of pyrogallol. His results failed to demonstrate the presence of CO<sub>2</sub> in the MEG though allowing for an experimental error the possible presence of less than 1% CO<sub>2</sub> could not be ruled out. In the MEG of man O<sub>2</sub> was present in the range of 14–17 volume per cent. Similar O<sub>2</sub> content was found in normal cats (12.8–16.2%).

Melvill Jones (1961) analysed the pressure changes in the ME of humans after simulated flights in a decompression chamber during breathing of known gas mixtures. By extrapolating the data he obtained values of normal near-equilibrium at approximately the 8% level corresponding to a 55 mm partial pressure of oxygen introduced into the middle ear for steady state condition. The partial pressure of CO<sub>2</sub> was estimated to be 40 mmHg. According to Drettner (1975) those results correspond to 9.3% O<sub>2</sub> and 5.5% CO<sub>2</sub>. However Melvill Jones (1961) emphasized that in view both of the indirect method of approach and the limited data available the above results cannot be considered a very reliable estimate.

Riu et al (1966) measured the human MEG composition by methods similar to ours namely gas chromatography of transtympanic MEG samples and found 9.5% O<sub>2</sub> and 5.5% CO<sub>2</sub>.

Sadé & Weisman (1977a) measured the CO<sub>2</sub> content of normal human MEG obtained after



Fig. 4 Base of the tongue of one of the agustic animals by Acetyl-Homocysteine. Observe the papillae intensely stained by osmium tetroxide

(from 9.52 to 12.03). In the rest of the organs the deviations of the values are not significant.

In the animals treated with D-Penicillamine in which ageusia did not appear the results were:

|        |                   |
|--------|-------------------|
| serum  | 8.44 $\gamma$ /ml |
| saliva | 18.78 $\gamma$ /g |
| liver  | 31.14 $\gamma$ /g |
| muscle | 14.77 $\gamma$ /g |

|        |                   |
|--------|-------------------|
| heart  | 11.27 $\gamma$ /g |
| gland  | 18.16 $\gamma$ /g |
| tongue | 8.60 $\gamma$ /g  |

The fall in copper concentration in the liver as in the case of Acetyl-homocysteine is important (from 56.80 to 31.14) (Fig. 1). The values for dispersion and variance are higher as far the liver is concerned (Figs. 2 and 3).

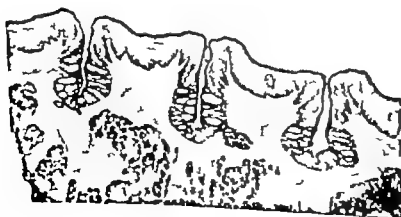


Fig. 5 Serial section of normal tongue of one of the agustic animals, by Acetyl-homocysteine

above the equivalent of 40 to 45 mmHg pO<sub>2</sub>. Melvill Jones (1961) found an early increase in the ME pressure during pure oxygen breathing in post flight experiments. Our analysis of MEG after 30 minutes of post anesthetic ventilation of dogs with pure oxygen has shown a significant increase in the oxygen concentration in the ME from 12 to 19% (Ostfeld 1979b).

## ZUSAMMENFASSUNG

Die Zusammensetzung der Gase im Mittelohr ist bei 5 normalen Hunden unter allgemeiner Anästhesie mittels Sodium Thiopentone untersucht wurden. Die Gasproben wurden durch Trommelfell Punktion gewonnen und gaschromatographisch analysiert. Folgende Gasmittelwerte  $\pm$  Standardabweichung wurden erhalten. N 83.2 $\pm$ 5.0%, O<sub>2</sub> 12.1 $\pm$ 2.2% und CO<sub>2</sub> 4.7 $\pm$ 0.7%.

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# HUMAN PITUITARY TUMOURS IN ORGAN CULTURE WITH CORRELATION TO CLINICAL DATA<sup>1</sup>

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**Abstract.** Out of 46 operated pituitary tumours 31 were hypersecreting growth hormone (GH), 12 hypersecreting prolactin (PRL), 2 hypersecreting ACTH and 11 tumours were chromophobe without secretion of hormone. One pituitary revealed normal conditions at the surgical plasmion later confirmed at the reserved endocrinological an enclaspation. 36 pituitary tumours have been explanted to *in vitro* culture. 20 tumours causing acromegaly, 9 prolactinomas, ACTH-producing and 5 chromophobe adenomas. The higher the GH concentration was *in vitro* the more active was the degree of acromegaly found clinically. GH hypersecreting tumours also secreted small amounts of PRL *in vitro* and the contrary concerning prolactinomas. The GH/PRL ratio was individual for each tumour. GH or PRL hypersecreting tumour tissue revealed approximately the same characteristics *in vitro* irrespective of whether taken from different regions of the tumour. A good correlation occurred in the secretion of PRL *in vivo* and *in vitro* the higher the serum level of PRL the higher concentration of PRL found in the culture medium. Mistakes did not occur in any of the histological sections of more than 400 separate specimens. The increase in PRL in cultured specimens is probably due to the absence of prolactin-inhibiting factor (PIF) during *in vitro* culture. On the other hand the decreasing GH levels may be due to the absence of GH-releasing factor. The results of the present study suggest an individual biology in each pituitary tumour.

Pituitary tumour disease presents a variety of signs and symptoms. Endocrinologically active tumours are nowadays generally diagnosed at an early stage, especially prolactinomas in women, but large bone-destructive lesions of the sella turcica have still not disappeared in clinical work. Such entities are encountered especially in growth hormone (GH) producing tumours and constitute a serious problem in the surgical treatment.

Medical treatment of pituitary tumours has been introduced in some cases of tumours hypersecreting prolactin (PRL) (Besser et al

1977, Varga et al 1977, Thorner et al 1974, Thorner & Besser 1978, Bergh et al 1978) and in a few cases of tumours causing acromegaly (Luzzati et al 1974, Thorner et al 1975, Besser et al 1976, 1978, Cassar et al 1977, Werner et al 1978). However the effect of this treatment with bromocriptine cannot be predicted and has to be tried in each case separately. The response to bromocriptine shows great individual variations, a fact that may later lead to surgery if the tumour grows during medication. A special problem is constituted by bromocriptine induced pregnancy in women with a known PRL hypersecreting tumour. The high oestrogen levels during pregnancy may induce a rapid tumour growth during this period of time (Griffith et al 1978, Besser & Moul, 1978).

At least a certain degree of an individual tumour biology may be suspected also in cases with the same type of hormone hypersecretion. The present study analysed GH and PRL hypersecreting pituitary tumours in an organ culture system—Independent of extra-pituitary regulatory mechanisms—in correlation with clinical observations of these tumours.

## MATERIAL AND METHODS

### Material

The transmaxillary or the transseptal route has been used for the exploration of the pituitary gland. The operating microscope was used during all surgical interventions of the sella

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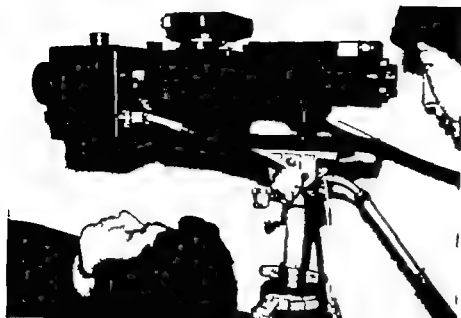


Fig. 1 Binocular infrared pupillograph in use.

attached to the camera makes it possible to take two photographs per second at a shutter speed of  $1/8$  sec. The depth of focus of this equipment is  $\pm 0.48$  mm, which is significantly smaller than the distance between the patient's eye and the film. Thus its magnification rate in the case of a well focused photograph of the pupil can be considered to be stable. Kodak HIE 420 was used as the film.

## MATERIALS AND METHODS

The patient was placed in a dark room where he rested for 10 minutes in a supine position. Then five photographs of the pupils were taken using the motor drive. The mecholyl test

was conducted *ad modum* Loewenfeld & Oono (1966). One drop of 5% mecholyl was instilled into the conjunctival sac of each eye under illumination and another drop 2 min later at which time the light was extinguished. Thirty minutes after the administration of the first drop five photographs were taken successively. The film negatives thus taken were placed under a binocular microscope ( $\times 20$ ), and the horizontal diameter of each eye was measured by the scale on the eyepiece (Fig. 3). The real diameter of the pupil was obtained by multiplying the above figures by a correction value of the magnification rate ( $\times 0.13$ ).

The pupil contraction rates were obtained in the following manner: pupil diameter 30 mm

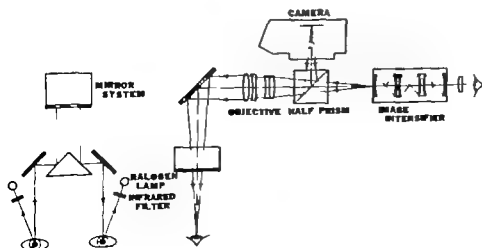


Fig. 2 Block diagram of the binocular infrared pupillograph.

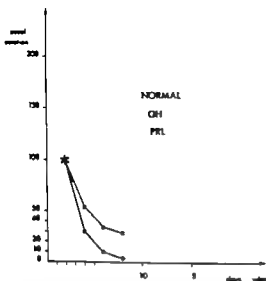


Fig. 1 Diagram illustrating the principles of GH and PRL synthesis/secretion from one normal pituitary gland *in vitro*. A difference is noted between GH and PRL secretion, the latter being more stable during organ culture but still decreasing with increasing time *in vitro*. The morphological processing revealed a large number of surviving cells in both groups of specimens.

## II GH hypersecreting tumours

In spite of the fairly similar tumour-induced hormone levels *in vivo*, considerable differences appeared when explanting tissue from individual tumours to the organ culture system. The GH secretion into the culture medium followed one of two main patterns, maintained hormone synthesis/secretion at a fairly constant level for several weeks in culture or a gradually decreasing GH secretion with increasing time *in vitro*. During the first week in culture specimens were occasionally found with an increased GH synthesis/secretion but the mode of secretion thereafter followed one of the two main patterns outlined above.

It was not possible to predict the *in vitro* synthesis/secretion of GH with regard to pre-operative GH levels. However, the GH concentration *in vitro* was well correlated with the clinical activity of the disease. With regard to the clinical activity of acromegaly the patients were placed in three different groups des-

Table III GH production *in vivo* and *in vitro*/clinical activity of the disease (acromegaly)

| Patient no      | GH in pmol/l                |                              | Clinical activity of acromegaly |
|-----------------|-----------------------------|------------------------------|---------------------------------|
|                 | <i>In vivo</i> <sup>a</sup> | <i>In vitro</i> <sup>b</sup> |                                 |
| 2               | 2 200                       | 3 900                        | +                               |
| 4               | 4 100                       | 3 200                        | +                               |
| 1               | 1 000                       | 3 300                        | +                               |
| 27              | 3 100                       | 1 670                        | +                               |
| 29              | 230                         | 750                          | +                               |
| 30              | 1 200                       | 3 680                        | +                               |
| 11              | 2 300                       | 2 950                        | +(+)                            |
| 1               | 1 200                       | 9 500                        | ++                              |
| 26              | 1 100                       | 4 100                        | ++                              |
| 31              | 5 800                       | 21 000                       | ++                              |
| 35              | 1 100                       | 18 500                       | ++                              |
| 3               | 1 100                       | 33 500                       | +++                             |
| 5               | 2 600                       | 35 000                       | +++                             |
| 6               | 2 800                       | 38 400                       | +++                             |
| 14              | 1 900                       | 900 000                      | +++                             |
| 17              | 4 000                       | 700 000                      | +++                             |
| 20 <sup>c</sup> | 230                         | 225 000                      | +++                             |

<sup>a</sup> Mean levels ( $n=6-10$ ) of fasting morning plasma GH.  
<sup>b</sup> Mean levels of *in vitro* GH secretion ( $n=8-10$ ) in pmol/48 h.

<sup>c</sup> Prior to surgery treated with bromocriptine for 3 months.

ignated +, ++ and +++ respectively, the latter indicating the most active degree of acromegalic disease. Independently of this classification the secretion of GH into the culture medium could also be grouped into three different classes. It was found that the more active the clinical disease was in the patient, the higher the GH concentration occurring *in vitro* (Table III).

All tumours causing acromegaly also secreted PRL *in vitro* in varying concentrations individual for each tumour but only as a minor part of the total hormone synthesis/secretion. The ratio of GH/PRL was the same in specimens ( $N=24-36$ ) from the same tumour but changed with increasing time *in vitro* (Tables IV and V). The synthesis/secretion of PRL in these tumours increased during the first 1-2 weeks in culture in approximately half of the tumours (Fig. 2). A few tumours revealed on the contrary a reduced secretion of PRL, whereas the remaining tumours had a fairly constant secretion of PRL into the culture medium.

Table II *The appearance of pathological miosis in normal and Meniere's disease groups*

The percentages in parentheses show the appearance rates of pathological miosis in each group

| Pupil contraction rate | Normal   | Meniere's disease |               |                    |               |                 |               |
|------------------------|----------|-------------------|---------------|--------------------|---------------|-----------------|---------------|
|                        |          | Interval stage    |               | Quasi-attack stage |               | Attack stage    |               |
|                        |          | Unaffected side   | Affected side | Unaffected side    | Affected side | Unaffected side | Affected side |
| <10%                   | 31       | 33                | 41            | 9                  | 8             | 4               | 3             |
| ≥10%                   | 1 (3.1%) | 8 (19.5%)         | 15 (26.8%)    | 2 (18.2%)          | 8 (50%)       | 2 (33.3%)       | 5 (62.5%)     |
| Totals                 | 32       | 41                | 56            | 11                 | 16            | 6               | 8             |

developed the condition while suffering from chronic otitis media and 3 after surgery of the middle ear. For the vestibular neuritis group 4 patients who could be tested within 2 weeks after the onset were chosen. The patients were also classified into attack, interval and quasi-attack stages depending on the severity of the vertigo at the time of the testing.

## RESULTS

The pupil contraction rates of normal subjects and Meniere's disease patients are shown in Table I. Although the rates among normal subjects were less than 14.9%, those of the patients were often higher, particularly on the affected side. Borgmann et al. (1974) in a study on the effects of 2.5% mechohyl on patients with pupillonia, stated that the pupil contraction rates of the unaffected eyes remained within 10%. Therefore a pupil contraction rate in excess of 10% was considered pathological and the rate of appearance of pathological miosis was obtained.



Fig. 4 The pupils before and after mechohyl application in a patient with right-sided labyrinthitis.

As shown in Table II the rate was 1/31 (3.1%) among normal subjects, whereas on the affected side of the Meniere's disease patients it was 5/8 (62.5%) at the attack stage, 8/16 (50%) at the quasi-attack stage and 15/56 (26.8%) at the interval stage. In the unaffected side among the patient group, however, these values declined to 2/6 (33.3%), 2/11 (18.2%) and 8/41 (19.5%) respectively. The rates on the affected side of Meniere's disease patients at each stage differed from that of normal subjects ( $P < 0.01$ ). On the other hand, no difference was found between normal subjects and the unaffected side of Meniere's disease patients ( $P > 0.05$ ).

Of the 5 patients with labyrinthitis, 2 had developed the disorder while suffering from chronic otitis media. These 2 patients were tested 10 days and 1½ months after their respective onsets. The remaining 3 had developed the condition secondary to stapedectomy for otosclerosis and radical mastoidectomy for otitis media. The mechohyl tests showed that no case had pathological miosis on the affected side alone. In one case, both eyes showed positive results (12.9% on the affected side and 12.2% on the normal side) and in another case, the eye on the normal side showed positive results. Fig. 4 is a photograph of the pupils before and after mechohyl application in the case which had the severest vertigo among this group at the time of the testing. The pupil contraction rate was 2.4% for the right eye and 7.2% for the left, thus not pathological.

tumours having a relatively stable GH and PRL secretion during at least one month in vitro. Although the preliminary experiments by Landolt et al (1979) indicate that hypothalamic factors are needed for long-term survival and growth of explanted human pituitary tumours this is likely to be the case only when extending the culture period over several months.

In prolactinomas there was a good correlation between the preoperative hormone level and the PRL synthesis/secretion into the culture medium in spite of the increasing synthesis/secretion of PRL with increasing time in vitro. This indicates a causality with regard to the biological activity of the tumour. Concerning GH hypersecreting tumours a causality is likely to occur between clinical activity of acromegalic disease and the in vitro level of GH.

Each pituitary tumour revealed individual variations with regard to parameters investigated in vitro. The biological behaviour of each tumour has therefore to be taken into consideration in the therapeutic management of the disease (acromegaly or hyperprolactinemia). Recently Anniko et al (1979) (to be published) demonstrated that the content of DNA in acromegaly varied according to the general aggressiveness of the tumour, i.e. clinical signs of activity, duration of the disease, bone destruction of the sella turcica etc. Highly active tumours showed an aneuploid content of DNA whereas clinically more benign tumours were diploid. It should be noted that cell mitosis is extremely uncommon as demonstrated in the present study. Although the DNA content varies among individual pituitary tumours the histological findings reveal a benign type of cell morphology.

There is little information on the effects of bromocriptine on the cellular or subcellular level of human pituitary tumours. Lloyd et al. (1975), von Werder et al. (1978) and others have claimed that bromocriptine has an antiproliferative effect—at least with respect to PRL hypersecreting tumours. Acromegalic

patients show a varying response to the drug in both acute trials and in long term treatment (Cassar et al. 1977).

Halves (1978) and others have reported progress of tumour growth during bromocriptine therapy concerning both GH and PRL hypersecreting tumours. Only a small number of cases have been published showing both a roentgenological regression of the pituitary tumour and a normalization of PRL levels following bromocriptine treatment (Valdya et al. 1977, Möhlenstedt et al. 1978). Recently Wass et al. (1979) published a series of 15 patients with PRL and 13 patients with GH hypersecreting tumours treated with bromocriptine only (prolactinomas 5–30 mg/day of bromocriptine for 8–78 months, acromegalic patients 10–60 mg/day of bromocriptine for 6–52 months). In the patient group having a PRL hypersecreting tumour 3/15 showed both roentgenological evidence of tumour shrinkage and a normalization of the serum hormone level. The patients with acromegaly had corresponding results in 2/13. It should be noted however that the acromegalic patients who showed roentgenological evidence of tumour regression were also hyperprolactinemic.

An early tissue culture study on the inhibitory effect of bromocriptine on PRL secretion was reported by Pasteels et al. (1971) (normal human pituitary gland). Subsequently Mashiter et al. (1977) and Adams et al. (1979) have confirmed these findings also as concerns pituitary tumours. Because of their technique (cell suspension) however it has been difficult to visualize the morphology of human tumour cells exposed to bromocriptine in vitro. In experimental animals bromocriptine-induced changes in fine structure were shown as long ago as 1972 by Ectors et al.

Tramu et al. (1979) reported that degenerative morphological changes had occurred in three operated cases of PRL hypersecreting tumours where PRL levels had returned to normal 13–17 months after commencing bromocriptine treatment. A correlation of



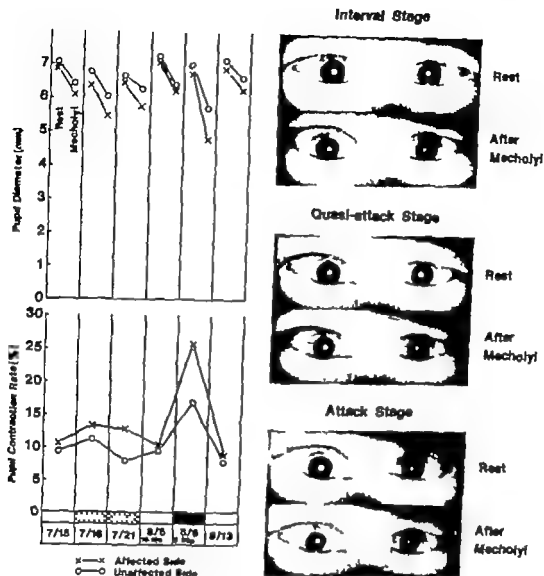


Fig 6 Case 2 Left-sided Meniere's disease showing left miosis after mecholyl application at the attack stage

### Case 2

A 53-year-old male with Meniere's disease on the left side was first seen on July 9 1976 because of his nine year history of episodic rotary vertigo accompanied by left sided hearing loss and tinnitus. In addition he experienced occasional attacks of a lateral swaying sensation of the body. Around 3 a.m. on July 16 1976 he started to have such an attack. The mecholyl test was conducted 9 hours later (quasi-attack stage). Again around 6 a.m. on July 21 the patient felt a similar dizziness and the mecholyl test was conducted 70 min later. During these quasi-attack stages spontaneous nystagmus toward the affected side was observed. Another mecholyl test was

conducted at 10 10 a.m. on August 6 during the interval stage. At this time there was creased tinnitus on the left side but the patient did not have vertigo. From 2 p.m. however he began to have a severe vertiginous attack during which he felt as if his surroundings were flowing to the right. The mecholyl test was started immediately. Spontaneous nystagmus toward the unaffected side was observed at this time (attack stage). The mecholyl test conducted at the interval and quasi-attack stages showed very little difference in pupil contraction rates of the left and right eyes. The test at the attack stage however showed strong miosis particularly on the left side (Fig. 6).



Fig. 1 Transverse section of the 2nd tracheal ring of male giraffe aged 15 years (VICTOR). Widest diameter 51 mm, shortest measurement 35 mm with tracheal length of 2.3 mm. Note the unequal lengths of the sides of the cartilaginous ring.

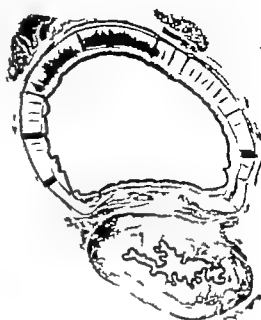


Fig. 2 Histological section of the tracheal ring shown in Fig. 1 showing the involuntary muscle lying posteriorly between the arms of the cartilaginous ring. This serves to diminish the tracheal lumen on expiration.

An assumption that these animals and others such as Deer, Antelope, Camels and Oxen are silent because they live in open country and can see each other ignores the need for purposeful vocal communication for survival. Cries for help occur even in otherwise silent animals such as the Hare and Rhinoceros who squeal when dying or in fear.

Warning of danger is of value for species survival and the sexual uses of sound are well known in the animal world including man. The use of panic as an important factor in spreading alarm by the giraffe—thereby scattering the herd and diminishing the safety of animal grouping, suggests that useful sound is impossible.

My continuing long term study of the detailed structure of the mammalian larynx (which now includes over 150 separate species) has provided an opportunity for examining the larynx and trachea of three specimens of giraffe. Attention has been paid to the problem of the large volume of tracheal dead space, conduction delay in recurrent laryngeal

nerves which may be 2.5 m long and laryngeal structure.

#### *Respiration in the Giraffe*

There is an important difference between respiration in ruminants and non-ruminating mammals. Rumen gas contains not only nitrogen but varying amounts of methane, oxygen and carbon dioxide. Rate of gas production is variable, possibly reaching 0.7 litres per minute but of considerable physiological importance is the amount of carbon dioxide produced; for some of this is inhaled and absorbed into the lungs (Patterson et al. 1965). Since mammalian respiration is designed to supply oxygen and remove carbon dioxide, inhalation of ruminant carbon dioxide will affect breathing patterns. Respiratory rates in giraffes in captivity have been noted by the author to vary from only 10 times a minute to over 45 times per minute when anxious. Long necks contain long tracheas and thus means a large respiratory dead-space. Attempts have

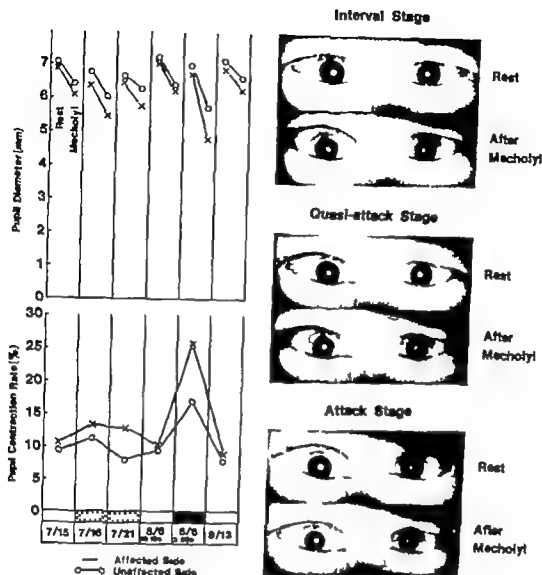


Fig 6 Case 2. Left sided Meniere's disease showing left miosis after mechohyl application at the attack stage

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ured in this manner are rarely circular in out-  
line and diameter was calculated as the diam-  
eter of the circle having a similar area to the  
fibre. Both recurrent nerves in the large 15-  
year-old male have been studied sections be-  
ing taken from the nerve as it passed beneath  
the inferior constrictor muscle. Although the  
nerve was approximately twice the size of that  
found in humans much of this was found to be  
composed of connective tissue. Total number  
of myelinated fibres was approximately 400  
with over 40% having a diameter greater than  
16  $\mu\text{m}$ . As yet the pararecurrent nerves have  
not been studied and it may be necessary to  
examine the superior laryngeal nerve as well  
to obtain a composite picture of the laryngeal  
motor nerve supply. Certainly this particular  
pair of recurrent laryngeal nerves appear to  
contain many less myelinated fibres than ex-  
pected although the proportion of large fast  
conducting nerves was greater than in the hu-  
man (Fig. 3).

Too great a significance must not be drawn  
from these findings for differences in the con-  
tractile characteristics of intrinsic laryngeal  
muscles may represent differences in the func-  
tional properties of their muscle fibres rather  
than variations in the diameter of their related  
motor nerve fibres. However the recurrent  
nerves of this particular giraffe did appear to  
contain a small number of fast conducting  
large myelinated nerve fibres.

#### *The Giraffe larynx*

Although a detailed account of the histological  
appearance of this structure will be given in a  
further publication Fig. 4 illustrates a macro-  
scopical view of the left half of an adult male  
animal. It bears some resemblance to that of  
the Horse possessing many of the features of a  
herbivore capable of swallowing semi-liquid  
food whilst continuing to breathe. The epiglot-  
tis lies above the soft palate and the large  
arytenoids surmounted by cartilages of Santorini  
protect the laryngeal inlet by means of the  
attached thyro-arytenoid folds. There appears  
to be additional support to these folds from

cartilages of Wrisberg. Attachment of the up-  
per end of the anterior wall of the oesophagus  
to the cartilages of Santorini help to open the  
former during deglutition (Figs 4 and 6). The  
flat thyro-arytenoid fold is approximately 25  
mm long and the slit above is shallow and  
probably does not represent a true division of  
the thyro-arythenoid muscle. In the fixed  
specimen it is difficult to determine whether  
the arytenoids are fully abducted. As in other  
ungulates they are hinged posteriorly produc-  
ing a diamond-shaped opening when the tips  
are abducted. In this specimen length of the  
arytenoids were 30 mm representing almost  
seven tenths the diameter of the glottis. Negus  
believed that this represented optimum condi-  
tions for glottic air flow and certainly the gi-  
raffe can equal the horse in a prolonged chase.

An animal requires only a simple larynx to  
produce sounds. The evolution of separate in-  
ferior thyro-arytenoid folds does not neces-  
sarily correlate with sound production for  
Bears with long, efficient folds use little voice  
neither does the Rhinoceros. However most  
animals make some definite purposeful sound  
under conditions of extreme stress and the  
Giraffe would appear an almost unique excep-  
tion. My investigations into the anatomical  
and physiological factors relating to respira-  
tion in this species suggests that this is not  
related to any unusual development in larynx  
or nerve supply. It is possible that the basic  
underlying deficiency lies in the inability of  
this animal to produce an air flow of sufficient  
velocity to vibrate the thyro-arytenoid folds or  
arytenoids. This difficulty may be enhanced  
by the presence of a contractile trachea which  
in the Horse necessitates sound production on  
inspiration.

#### ZUSAMMENFASSUNG

Trotz Besitzen einer gut entwickelten Larynx und einer  
sehr gewiesigen Natur kann die Giraffe nur ganz leise  
Bölen oder Röhren. Nach morphologischer und histolo-  
gischer Untersuchung, sowie Messungen der Luftströme  
und subglottisches Gefälle von drei frischen Larynxen  
(Giraffe *Camelopardalis*) konnten wir versuchen die Ab-  
wesenheit einer adäquaten vokalen Fähigkeit erklären.

the section of the nerve but takes a certain amount of time as reported in cases of denervated pupillary sphincter in the cat (Shen & Cannon 1936; Keil & Root 1941).

However the question has been raised as to whether the appearance of significant miosis is due merely to parasympathetic paralysis. We have observed that the mechohyl test caused remarkable miosis in the affected eye of a patient with peripheral Horner's syndrome. As regards the site of the underlying lesion it should be noted that in Adie's syndrome indicating characteristic supersensitivity to mechohyl the pathology was thought not to be situated in the periphery but centrally in the region of the hypothalamic vegetative centers (de Haas 1959). Therefore at present positive results in the mechohyl test should be interpreted as an indication of the existence of either peripheral or central autonomic dysfunction on the same side.

There has also been some question as to whether the autonomic dysfunction observed in patients with Meniere's disease especially at the attack stage is directly related to the cause of the disease or not. This is because changes in the peripheral labyrinthine function may induce autonomic dysfunction secondarily through the vestibular-autonomic reflex.

Of the 4 patients in which significant miosis was observed on the affected side during the attack stage 2 were tested within two hours of the onset the remaining 2 during the attack or 5 minutes prior to it. This may indicate the existence of autonomic dysfunction as the cause of the attack. However the references on the time required for the development of supersensitivity have been obtained through animal experiments alone and observations on human subjects are expected in this connection in the future.

In order to solve this question the test results were compared with those obtained with patients suffering from labyrinthitis and vestibular neuritis in whom a labyrinthine disorder was caused by the disease. No one showed positive results limited to the affected

side. This eliminates the possibility a labyrinthine disorder alone can cause a positive reaction of the affected side in the mechohyl test. Therefore we can conclude that autonomic dysfunction revealed by mechohyl tests in patients with Meniere's disease be present as a cause of the disease and secondary to the occurrence of a labyrinthine disorder.

## ZUSAMMENFASSUNG

Um die Existenz einer vegetativen Dysfunktion als Ursache der Meniereschen Krankheit festzustellen, 100 Mechohyl Tests mittels Bindehautanästhesie von 100 Mechohyl normalen sowie bei an Menierescher Krankheit, an Labyrinthitis und an vestibulärer Neuritis leidenden Patienten durch. 1) Die Frequenz einer signifikanten Pupillenverengung (größer als 10%) war bei Normalen 31% signifikant höher bei den Meniere Patienten an d. betroffenen Körperseite im Anfalls-Quasiattacken-Intervallstadium. Die Häufigkeit im kontralateralen der Meniere Patienten war im keinem der drei Stadien anders als bei Normalen. 2) Keiner der Patienten mit Labyrinthitis oder vestibulärer Neuritis zeigte eine signifikante Miosis der involvierten Seite allein im Mechohyl Test bedeutet, daß ein abnormaler vestibulär-autonomer Reflex an sich zu keinem positiven Mechohyl Test. Es kann daher geschlossen werden, daß die Ursache der Meniereschen Krankheit mit der Existenz einer vegetativen Dysfunktion der betroffenen Seite im Zusammenhang steht.

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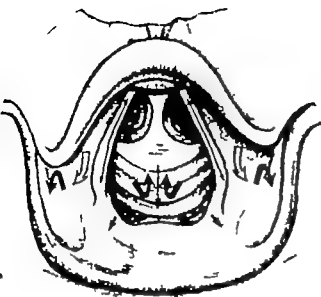


Fig. 2. Lymphatic drainage of the inner larynx: posterior aspect.

#### Electron microscopy

As during lymphangiography a morphological classification into lymph capillaries and collecting vessels is feasible. The lymph capillaries show a continuous thin endothelial layer with thickening in the nuclear area. The nucleus shows a double layered membrane with pores and a perinuclear cisterna. Within the cytoplasm there are mitochondria and many mostly longitudinally orientated fine filaments. There are multiple luminal and ab-luminal invaginations of the endothelial membrane and numerous smaller and larger vesicles within the cytoplasm. These vesicles seem to cross the cell similar to microphocytosis. The endothelial cells are overlapping sometimes for a distance of a few microns with intercellular gaps measuring between 30 Å and 0.5 µm. When the junctions are pulled apart there is a rather free communication between the capillary lumen and the interstitial space.

The lymphatic collecting vessels show similar structural components except for two major characteristics. There is a collagenous adventitial layer with elastic respectively con-

tractile elements and there are valves within the lumen. These valves are covered with an endothelium and the stroma is made out of loose connective tissue with collagenous and elastic fibres as well as fibroblasts.

#### DISCUSSION

Our experiments by means of lymphangiography and ultrastructural studies show that laryngeal lymphatics may be divided into lymph capillaries and collecting vessels. The function of this lymphatic system is to drain the interstitial space and by means of lymph angioscopy the drainage mechanism can be observed over longer periods. There are remarkable differences between in vivo and post mortem studies because in the latter case the dissemination of the dye depot in the interstitial space leads to a false compartmentation due to interstitial connective tissue bands.

Our lymphangiographic studies of the inner larynx demonstrate a sector-like centripetal lymph drainage via lymphatic collecting vessels into the regional lymph nodes. As multiple collaterals at the capillary and the collecting

## MENIERE'S DISEASE AND THE MASTOID PNEUMATIZATION

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**Abstract.** The size of the mastoid pneumatization was radiographically examined and planimetrically measured in the patients with Meniere's disease, patients with other sensorineural hearing loss and clinically ear healthy persons. Law projection was used for the radiographical examination. No statistical differences were seen among the three groups. Meniere's disease and the factors which influence the mastoid pneumatization are discussed.

The aetiology of Meniere's disease is still unknown. However, the histopathological finding is a distension of the endolymphatic compartments, especially the cochlear duct and succule, due to an increase in the volume of endolymph (hydrops). According to Schuknecht (1974), atrophy of the cochlear neurons of the apical turn, vestibular fibrosis and epithelial degeneration in the stria vascularis are other observations.

Concerning the pathogenesis of endolymphatic hydrops, the endolymphatic sac and vestibular aqueduct have been shown to play an important role (Yuen & Schuknecht 1972; Stahle & Wilbrand 1974; Arenberg et al 1977). The radiographical abnormality of the vestibular aqueduct, which contains the endolymphatic duct and part of the sac, was reported by Clemens & Valvassori in 1968 and they suggested a correlation between an invisible aqueduct and Meniere's disease. Wilbrand et al (1978) suggested that periaqueductal pneumatization is sometimes poor or absent in the affected area of Meniere's disease.

The aim of this study was to discover the total pneumatization of the temporal bones among the patients with Meniere's disease, patients with other sensorineural hearing loss

and clinically middle ear healthy persons and show whether or not statistical differences are seen among these three groups.

### MATERIALS AND METHODS

Twenty-seven patients with Meniere's disease (16 males aged 23-55, 11 females aged 21-72), 27 patients with other sensorineural hearing loss (16 males aged 21-58, 11 females aged 28-74) and 25 clinically healthy persons (13 males aged 20-56, 12 females aged 20-58) were radiographically examined. Of the 27 patients with Meniere's disease, 10 males had bilaterally affected ears. Not included with the patients with other sensorineural hearing loss were patients with sudden deafness, congenital syphilis, perforated eardrums, anamnesis of acute or chronic otitis media or anamnesis of tuberculosis.

Law projection was used for the radiographical examination. The size of the mastoid air cell system ( $\text{cm}^3$ ) was measured by a planimeter. The space of the middle ear was excluded from the measurement.

### RESULTS

The size of the mastoid air cell system in the patients with Meniere's disease, patients with other sensorineural hearing loss and clinically ear healthy persons are shown in Table 1. No statistical differences were seen among Meniere's diseased persons and those having sensorineural hearing loss or those clinically

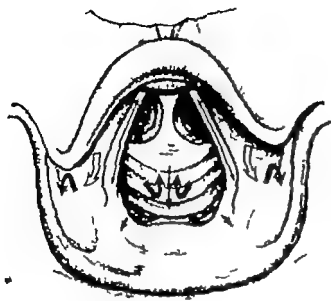


Fig. 2 Lymphatic drainage of the inner larynx, posterior aspect

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cal factors for Meniere's disease or not by our radiographical examination of the temporal bone.

## RÉSUMÉ

La taille des cellules pneumatiques du système mastoïdien a été examinée radiographiquement et mesurée par planimétrie chez des malades atteints de maladie de Menière de perte d'audition neurosensorielle et sur des sujets dont les oreilles étaient saines. La projection de Law était utilisée pour un examen radiographique. Aucune différence statistiquement significative n'était trouvée parmi ces trois groupes. La maladie de Menière et les facteurs qui influeraient sur la pneumatisation mastoïdienne sont discutés.

## ZUSAMMENFASSUNG

Die Größe der mastoideen Pneumatisation wurde radiographisch untersucht und planimetrisch gemessen in den Patienten mit Menierescher Krankheit, den Patienten mit anderer empfindungsnervlicher Gehörlosigkeit und bei Personen mit klinisch gesunden Ohren. Lawprojektion wurde für die radiographische Untersuchung angewandt. Keine statistischen Verschiedenheiten waren unter diesen drei Gruppen gemerkt. Die Menieresche Krankheit und die Faktoren die auf die mastoide Pneumatisation einwirken werden erörtert.

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segmentally directed by valves taking the way of least resistance. In case of tumorous or infectious blockade or valvular insufficiency the segmental drainage is abolished and alternative routes lead to involvement of different lymphatic areas.

## ZUSAMMENFASSUNG

Lymphangiographische und elektronenmikroskopische Untersuchungen lassen am Kehlkopf eine Gliederung des Lymphgefäßsystems in Lymphkapillaren und Lymphknotengefäße erkennen. Die letzteren unterscheiden sich von den Lymphkapillaren durch das Vorhandensein von Klappen sowie durch eine elastische Gefäßwand. Die Lymphangiographie zeigt, daß eine strenge Kompartimentierung einzelner Kehlkopfregionen nicht besteht. Anstatt dessen bilden die Lymphkapillaren des freien Randes der Stimmlippen Anastomosen der Lymphkapillaren und Kollateralkapillaren des Kehlkopfes und Kollateralkapillaren des Kehlkopfes. Diese Kollateralkapillaren können bei Behinderung der physiologischen Lymphdrainage eine besondere Bedeutung zu

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## DISCUSSION

*Answer to Beck.* We should be grateful to Mr Beck because through his observations, he has made realize areas the possible risks of partial larynx resections. The existence of compartments and then of anatomically proved boundaries for the expanding tumor in the soft parts of the larynx nevertheless play minor part in the indication of the critical partial resections. However there is a different morphological basis for the principle of horizontal partial resections. I may recall that it was the clinical observations of the roentgenologist Barlesse which stimulated J. M. Alonso to develop his surgical concept of the horizontal partial resection. I may further bring to mind that embryologically speaking the larynx is developed by a cranial and a caudal part of which is later the wedge coming together at the level of the laryngeal ventricle. This leads to the area of fusion to kind of watershed and thus also to an effective barrier against cancerous growth. It would be very bad for numerous patients if the result shown by Mr Beck were to come quick to lead to mislead of partial resectioning of the larynx. The quality of life of patient who has had successful partial resection is incomparably better than that of patient who has had total laryngectomy. The formation of a neoglottis (Steffen, Amann) undoubtedly hardly changes anything here. Partial resections of the larynx are often so helpful that their justification is beyond doubt as far as I am concerned. However their success depends greatly on very strict indication. This also is illustrated by M. Beck, emth.

*Ekrenberger to Beck.* Können Sie die Angaben anderer Autoren bestätigen, dass die Membrana hyopiglottica eine strikte horizontale Grenze zwischen der Lymphgefäßversorgung des Zungenknorpels und des präepiglottischen Raumes darstellt?

*Julius to Beck.* Unter 1800 Henslaryngektomien erreichten wir 80% Fünfjahresheilungen. Nur wenige Patienten zogen zu Totalresection. Wie können die Differenzen zwischen den Untersuchungen der Lymphgefäße und den klinischen Erfahrungen erklärt werden?

*Klaus to Beck.* This paper is very interesting for people doing partial surgery on the larynx. I would like to make two remarks.

Doing the partial surgery of the larynx it is necessary to

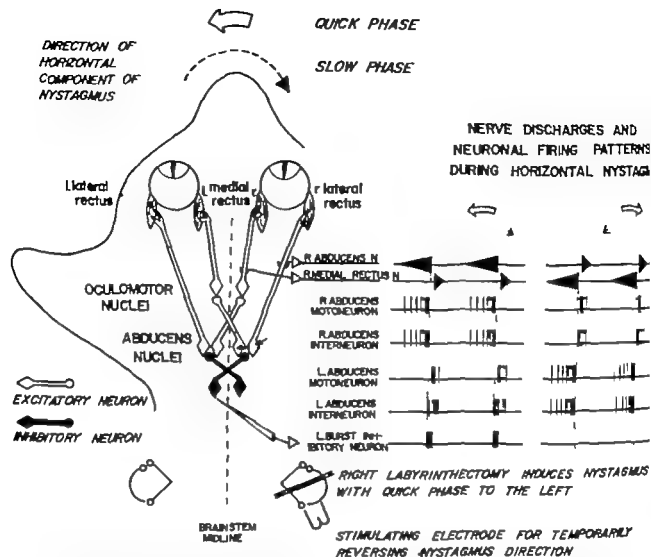


Fig. 1 Schematic representation of some of the anatomical connections involved in horizontal eye movement control. The neural firing patterns of these elements during horizontal vestibular nystagmus are shown on the right. The connections and firing patterns shown schematically here are derived from previous physiological studies of

abducens motoneurons and interneurons (Baker & Highstein 1975; Maeda et al. 1972; Nakao & Sasaki 1978) and burst inhibitory neurons (Hikosaka & Kawakami 1977; Hikosaka et al. 1978b). The italicized letters describe the procedures in the present experiment.

activity of abducens motoneurons is brought about by the action of a small group of inhibitory neurons located around the caudal pole of the contralateral abducens nucleus (Hikosaka & Kawakami 1977). These neurons have been called burst inhibitory neurons (BINs) and we shall use that term. BINs uniformly exhibit a short burst of high frequency firing (up to 800/sec) specifically at the end of the slow phase of horizontal nystagmus only when the slow phase is directed to the contralateral side. They are silent or only fire sporadically during nystagmus in the opposite di-

rection. BINs are inhibitory and terminate on motoneurons in the contralateral abducens nucleus (Hikosaka et al. 1978b). Consequently the burst of firing of these premotor neurons acts to terminate the slow phase activity of contralateral abducens motoneurons.

Other cells in the pontine reticular formation have been shown to fire in relation to rapid eye movements (Luschei & Fuchs 1972; Keller 1974; Henn & Cohen 1976; Cohen & Henn 1972). However the exact role of these cells in the generation of rapid eye movements remains to be established. BINs were spe-

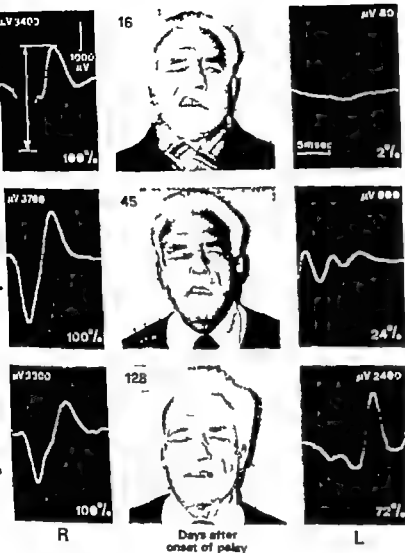


Fig 2 Electroneurogram of patient with facial palsy after parotidectomy

placed at the point where the facial nerve emerges from the stylomastoid foramen. Its two poles are set between the apex of the mastoid and the condyloid process of the mandible and in front of the tragus respectively.

The surface bipolar recording electrode is attached to the nasolabial sulcus and connected to the Mk III preamplifier. The test is carried out in a Faradayised booth though this precaution is not absolutely necessary.

A comparison is made between the responses of the unimpaired and injured sides to stimuli of the same intensity. A percentage esti-

mate can thus be made of the number of fibres able to produce muscle contractions on the injured side.

The test is both quick and easy to perform. Patient tolerance is good. The final response consists of a roughly sinusoid wave whose positive-negative peak amplitude can be very accurately measured in mV to give the percentages required. Like all previous Authors in this field we evaluate the amplitude of the potential only. Latency and the area of the wave are of scant diagnostic and prognostic interest.

lution saturated with Fast Green FCF (12 M ohm in resistance) were used for extracellular recording of unit spikes. EEG was amplified with an AC (0.3 sec time constant) differential amplifier. Extracellular unit spikes and compound action potentials of abducens and medial rectus nerves were recorded with single-ended AC amplifiers having 1.0 msec time constants. These potentials were fed to an oscilloscope (Tektronix Type 5112) and when necessary recorded simultaneously on moving film magnetic tape and a strip chart recorder. A comparator one-shot circuit was used to change the unit spikes into idealized spikes. Firing rate was measured by low pass filtering of the idealized spikes or by a frequency to voltage converter (Curthoys 1978). A four-channel strip chart DC recorder (Gould Brush 2400) run usually at 25 mm/sec recorded BIN firing rate together with EEG abducens and medial rectus nerve discharges. In some cases blood pressure was recorded instead of medial rectus nerve discharge.

The anesthetic agent employed was 20% nitrous oxide in 80% oxygen. Two respirators were used during the experiment. The one already mentioned maintained the animal on normal room air. Another respirator was connected through a Sweetman anesthetic machine for delivery of the nitrous oxide when necessary. After recording in the pre-anesthetic state for control, the endotracheal tube was changed to the nitrous oxide delivery system which had been pre-charged with the above concentration of nitrous oxide to minimize dead air space. The anesthetic was discontinued either when the burst firing of brain stem neurons became tonic or stopped or after 4–6 min of administration. After recovery from anesthesia had been confirmed by EEG patterns, the next neuron was studied with the same procedure. Thus records of 2 to 4 neurons were usually obtained from each experiment. The volume and rate of respirations were not changed before or during anesthesia. Normal physiological condition of the cat during the experiment was checked by en-

suring that the concentration of  $\text{CO}_2$  in the expired air remained at around 4% using a Beckman  $\text{CO}_2$  analyzer.

Recording sites of brain stem neurons were marked by electrophoretic ejection of Fast Green FCF (20  $\mu\text{A}$  for 10 min) through the recording microelectrode (Thomas & Wilson, 1965). At the end of each experiment, the animal was sacrificed by intravenous injection of a lethal dose of pentobarbital and the brain stem was fixed in 10% of formalin solution. Frozen sections 50 micrometers thick were stained with Nissl-staining preparations.

## RESULTS

### Identification of BINs

Unit spikes were recorded extracellularly from the reticular formation in the vicinity of the abducens nucleus. Neurons identified by the following characteristics were considered to be BINs. (1) They were found in the dorso-medial part of the reticular formation at the level of or just caudal to the abducens nucleus and slightly lateral or inferior to the medial longitudinal fasciculus (MLF). (2) They exhibited bursts of firing exclusively at the quick phase of vestibular nystagmus directed to the ipsilateral side. (3) They were silent or fired sporadically during nystagmus with quick phase in the opposite direction (see Fig. 1, Fig. 2). This identification was based on the findings of Hikosaka et al. (1977, 1978b). These criteria excluded the possibility of recording other nystagmus-related neurons such as abducens motoneurons and interneuronal neurons of the abducens nucleus and neurons in the prepositus hypoglossi nucleus and adjacent reticular structures which unlike BINs fire with a burst/tonic pattern during both directions of nystagmus (Hikosaka et al. 1978a; Maeda et al. 1972; Nakao & Sasaki 1978).

Sixty five neurons which satisfied these criteria were recorded. Of these 30 neurons whose spike potentials were stable and well

of urgency. Rapid, efficient and dependable diagnostic techniques are essential therefore.

Electroneuronography is able to satisfy these requirements in a particularly effective manner. By comparison with other electrodiagnostic methods it has the prime advantage of offering early exact data that can be readily translated into quantitative values. If the nerve deficiency is more than 90% for example surgery may be undertaken less than 72 hours after the onset of traumatic paralysis since the simplicity and rapid execution of the test enables what amounts to a continuous watch to be kept on any progressive degeneration of the nerve.

Evaluation of course will depend on the aetiology. However once it is remembered that a facial defect does not become clinically evident until more than 40% of fibres cease to function, a test capable of detecting changes of only 5% is clearly a valuable guide in assessing both the further progress of a paralysis and the effects of treatment.

False negatives or positives are of course inevitable in all forms of diagnostic enquiry. Decisions with respect to treatment therefore should definitely be taken in the light of the evidence provided by several examinations, which should be repeated in further sessions wherever possible.

## ZUSAMMENFASSUNG

Die Elektro-neuronographie (ENOG) besteht in der Aufzeichnung eines durch einen eigenemachen elektrischen Reiz hervorgerufenen Somatosensationspotentials der motorischen Einheit. Die Depolarisation (und offensichtlich die Unterbrechung) einer Nervenfaser bedeutet immer die Denervierung aller sich auf dieselben beziehenden Muskelzellen, so daß die Aufzeichnungsabnahme des Somatosensationspotentials der Anzahl der efferenten motorischen Einheiten verhältnismäßig sehr wird. Dem Test liegt der Vergleich der Gegenreaktionen, je Reiz gleicher Stärke, zwischen gesunder und kranker Seite zugrunde. Wie ermöglicht, die Anzahl der Nervenfaser des verletzten Teils unmittelbar festzustellen, die gegenüber dem unbeschädigten Teil vermindert, das Zusammenstoßen der deren abhängigen Muskelzellen hervorgerufen. Der Verfasser schildert die bei 60 persönlich beobachteten Fällen erhalten Ergebnisse und bezieht die neurologischen System geborenen Defekte.

seiner Vorteile bei der Beurteilung der Entwicklung einer Paralyse des Gesichtsnervs und bei der Festlegung genauer und vorzeitiger therapeutischer Angaben vor allem von chirurgischen Gesichtspunkten aus.

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## DISCUSSION

*Stable to Rossi:* Have you any experience of measuring the area of the entire reaction besides the amplitude?

*Jakob to Rossi:* Die guten Erfahrungen können wir aus bestätigen, nämlich aber ist III. Portion, das mehrere Parameter zur Beurteilung eines Verlaufes der Facialisparese notwendig sind. Der Fingerdruck, mit dem Rossi die Elektroden haben, kann können eine Fehlerquelle sein. Wir bevorzugen deshalb für unsere sehr viel kleineren Elektroden den Bernstoffkleber.

*Pfaff to Rossi:* A single response in electroneuronography is not very reliable. For this reason, repeated responses ought to be used for more reliable evaluation of the global facial response.

*Ehrenberger to Rossi:* Après nos expériences la neuroneuronographie est une méthode éprouvée en cas des paralysies de Bell mais pas en cas des paralysies traumatiques même une partie totale de la fonction perdant la première semaine peut être suivie d'une restauration totale de la fonction normale après plusieurs semaines.

*Rossi (Reply):*

Electroneuronography is a very useful test in the diagnosis of facial palsy. Yet it is by no means the only electrical

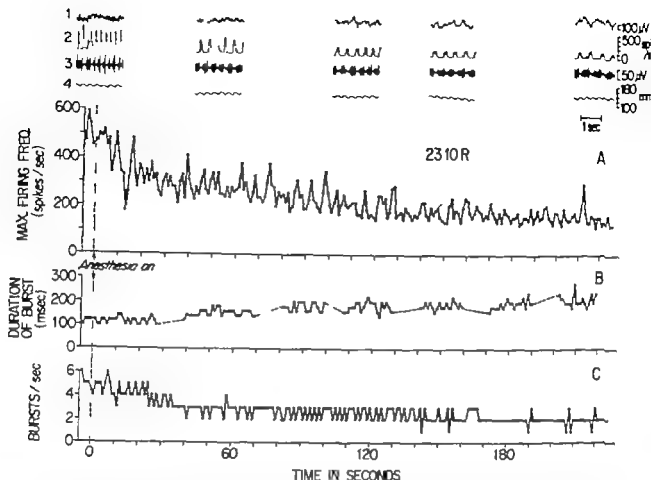


Fig. 3 Changes of burst activity of a BIN in the early stage of anesthesia. (A) Maximum firing frequency refers to the maximum firing rate of the bursts in each 1 sec interval. (B) Duration of the burst in A. (C) Number of bursts in each 1 sec. Time scale for C applies to A and

B. Upper inset figures are traces of EEG (1) firing rate of the BIN (2) contralateral abducens nerve discharge (3) and arterial blood pressure (4) recorded simultaneously by the strip chart recorder. Each set of the records is obtained at the time indicated in the abscissa of C.

lowed by a rapid decrease (A 3). At the end of the slow phase the BIN exhibited a high frequency burst of firing (sometimes more than 600 spikes/sec in the initial part of each burst) lasting 60–90 msec in duration (A 1). The onset of each burst approximately coincided with the time of abrupt cessation of abducens nerve discharge and preceded by 5–10 msec the sudden production of medial rectus nerve discharge. When the direction of nystagmus was reversed by high frequency electrical stimulation of the vestibular nerve on the side of labyrinthectomy (B 2 and 3) the burst discharges of the BIN neuron were absent and individual spikes were rare (B 1).

In alert animals before administration of the anesthetic the maximum firing rate of BINs ranged from 220 to 880 spikes/sec and this

peak rate occurred typically in the first 5 to 8 msec of each burst. The duration of each burst was from 60 to 140 msec. The number of bursts which corresponds to the number of nystagmic beats ranged from 2 to 4.5/sec.

We wish to emphasize the uniform powerful inhibitory effect of BINs on contralateral abducens motoneurons. BINs are typically silent at rest but during recovery from anesthesia, they fire tonically at a gradually increasing rate before returning to the pre anesthetic mode of short bursts separated by silence. During this time of tonic BIN firing the activity of the entire contralateral abducens nerve was found to be governed by the activity of BIN cells. Fig. 2D shows two cases where as the firing of just one BIN varies so the discharge of the entire contralateral abducens nerves varies in

## THE R THRESHOLD OF THE QUICK COMPONENT

*A New Measure in Electronystagmography*

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Vestibular imbalance affects the eyes through the fasciculus longitudinalis medialis and oculomotor nuclei. As regards vestibular excitation eyes could theoretically be turned around and around until balance is achieved. However, since physical and anatomical limitations do not allow such turning around, the eyes could be held in the utmost lateral position in the position of conjugated deviation. Nevertheless, the eyes are not held in this position of conjugated deviation since the compensatory central component brings the eyes back to the position of azimuth zero. Logically, this should depend on physical limitations and on the tension of the eye muscles which transmit information to the central structures via the proprioceptive afferent paths. The speed of the slow component and other elements also play a certain role here. It seems that we are faced with a great task of determining all these elements in order to define the point where the returning quick eye movement begins.

There are many elements which send commands to revert eyes. Sometimes the eye reverts after a high amplitude and sometimes after a low amplitude. It is not the same in all individuals (Collard & Eber, 1977). Many central mechanisms which are involved are not yet fully understood. The number of the unknown elements is too large to allow any possibility of their definition and measurement (Fig. 1).

However, the first step was carried out by determining the point where the slow component of nystagmus stops and the quick component begins as the point where the mechanism for reverting the eye starts to work. This point

could be called the threshold of the quick component. This means that the point is identified but not explained. Its identification makes it a possible subject for investigation.

*Identification and Measurement of R*

The assumption that this point can be defined only by knowing all the elements which determine it, is quite discouraging. It is possible, however, to make a comparison between the many elements of nystagmus which are measurable even though their mechanisms are still not well known. We may say that it is not absolutely necessary to know all the mechanisms in order to determine and measure this threshold. On the contrary, by making the threshold of the quick component measurable, it serves for purposes of investigation and learning about the elements which determine it.

The next step in our exploration was made when we assumed that if there is a certain threshold of sensitivity for the appearance of the quick component, then it must be constant in the examined individual, no matter whether nystagmus is weak or strong, whether it is at the beginning, in the middle or at the end of the provoked nystagmus. This is called the law of preservation. The Nobel Prize winner physicist Feynman says to a physicist, the law of preservation means the existence of a number which is definable at a certain moment and if we define it again after some time the number will not change—it will be of the same value, no matter what changes occurred in the meantime.

The threshold of the quick component was



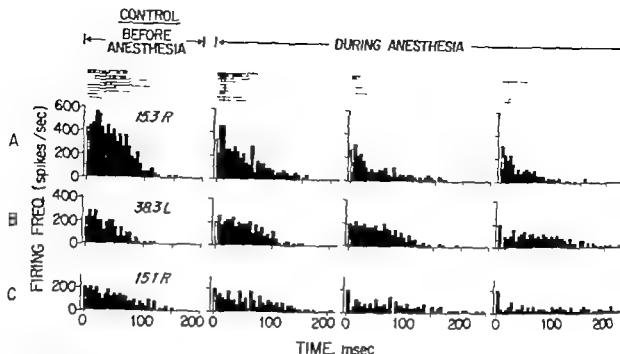


Fig. 4 Histograms showing detailed changes in the characteristics of the burst for three BINs during anesthesia. (A) Each histogram was constructed by measurement in each 5 msec and averaging of dot plots of spike discharges of 10 successive bursts shown above it. The first histogram during anesthesia was made from 10 successive

bursts beginning 20 sec after the onset of anesthesia. Intervals of 20 sec elapsed between the later bursts. The time zero in each abscissa indicates the onset burst. The hatched bin shows the artificial control (see text). (B, C) Constructed from other neurons.

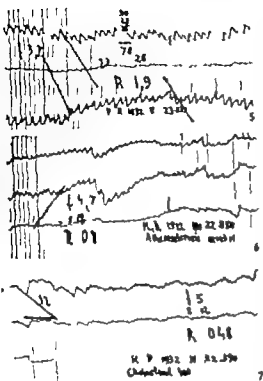
from 10 to 240 sec. The mean time was  $64 \text{ sec} \pm 54 \text{ (S.D.)}$ ,  $n=22$ . The time to the first detectable change in abducens nerve discharge (usually a decrease in amplitude or rounding of the end of the slow phase) ranged from 15 to 240 sec (mean  $62 \pm 53$ ,  $n=22$ ). Similarly the time to the first detectable change in medial rectus nerve discharge ranged from 11 to 150 sec (mean  $62 \pm 49$ ,  $n=13$ ). Changes in the BIN abducens and medial rectus nerve activity occurred at about the same time as the first detectable slowing of EEG (range of 15 to 240 sec, mean  $74 \pm 54$ ,  $n=22$ ).

Fig. 4 shows more detailed changes in the characteristics of the BIN burst for three neurons during anesthesia. Each firing rate histogram was constructed by measuring 10 successive bursts aligned at the first spike as shown by the dot plots for 153R at the top of the figure. The average number of spikes in each 5 msec interval was multiplied by 20 to give the average firing rate in that interval. The hatched vertical bar at the left of each histo-

gram represents the artifactual addition of firing rate resulting from using the first burst for alignment. Before anesthesia the firing rate of all three neurons reached a maximum in the initial 10–30 msec of the bursts and gradually decreased. One neuron retained its burst pattern during anesthesia although the firing rate progressively decreased (A). In the other two neurons the time to the maximum firing rate was prolonged with a progressive decrease in firing rate and increase in the burst duration during anesthesia (B and C).

#### The later stages of anesthesia

After longer periods of nitrous oxide administration, slow waves of 2–3/sec and/or a slow wave appeared in the EEG (Fig. 6A1). In the BINs their regularly repeated bursts were replaced by tonic firing, with further an increase in firing rate. During anesthesia, this tonic firing gradually decreased to zero (Fig. 6A2). Immediately before this tonic firing the maximum firing rate of bursts ranged from 50 to 150 spikes/sec; the burst duration



Figs. 5-7 5 A case of atherosclerosis, with  $R=1.9$  6 Another case of central atherosclerosis ( $R=0.3$ ) 7 A patient whose first measured  $R$  value was 0.3. It was subsequently found that his cholesterol value was 400

the illness progressed  $R$  increased to reach 64 in few months.

#### Angiopathies and R

In our investigations we noticed that the value of the threshold of the quick component decreases in central angiopathies. It is less than 2 and in greater disturbances it is less than 1 (Figs. 5-7).

#### Possibilities open to Investigation

Our investigations until now were limited only to the following two points: (1) description and use of the new element in the electronystagmogram viz. the threshold of the quick component, and (2) explanation of the possibility of measuring this threshold and expressing its value numerically.

Everything else presented in this commun-

cation is only the beginning of the work which in our opinion promises significant diagnostic possibilities.

As an example for investigation we would like to mention a formula for fixational suppression

$$FS = \frac{NF(\text{non-fixation}) - F(\text{fixation})}{NF(\text{non-fixation})}$$

where usually the speed of the slow component is worked with but it is also possible to use the threshold of the quick component  $R$ . We did it while examining a few healthy individuals and obtained the same values for the fixational suppression in  $s$  and  $R$ .

Fixational suppression expressed in  $s$  was 85.1 and in  $R$  it was 85.7. It is interesting because in some pathological cases regarding fixational suppression there was no connection between values of the speed of slow component and the threshold of the quick component. It means that it is possible to discover disturbances in some mechanisms of fixational suppression and surely there is more than one.

It is easy to construct a formula for calculating  $R$  in each individual nystagmic jerk. We adjusted it to the speed of the ENG sheet of 15 mm/sec while for the speed of 10 mm/sec we should substitute the number 10 for 15.

$$R = \frac{S}{\left(\frac{15}{N}\right)} \quad \text{or} \quad R = \frac{S}{\left(\frac{10}{N}\right)}$$

$N$  = length of the nystagmic jerk, in mm

$$\frac{15}{N} \quad \text{or} \quad \frac{10}{N} = \text{frequency of 1 jerk}$$

This is a new datum which can help in the exploration of nystagmic irregularities where the change in threshold of the quick component is mostly involved.

Various influences and effects on the threshold of the quick component can be explored: effect of sedatives, influence of the various levels of intelligence and motorics relationship with neurological diseases especially

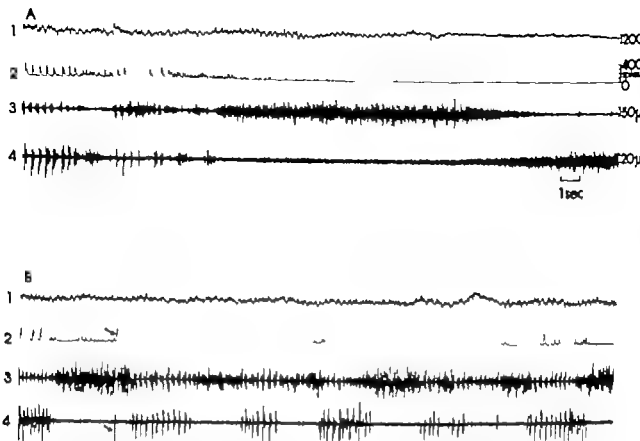


Fig. 6 Change from burst to tonic firing of a BIN and its complete reduction of firing in the later stage of anesthesia. Simultaneous recording of EEG (1) BIN firing rate (2) and the activity of the contralateral abducens (3) and medial rectus nerves (4) during anesthesia. (B) To show the close relationship between BIN firing rate and medial

rectus discharge. The fluctuations in BIN firing is matched by fluctuations in the medial rectus discharge even to the extent that one BIN burst with a high firing rate (arrow) is accompanied by a medial rectus discharge. Same arrangement and calibrations as in A.

### Medial rectus discharge

One surprising result was a high correlation between the firing rate of single BINs and the strength of the medial rectus discharge at the quick return phase. This correlation was usually observed in both unanesthetized and anesthetized animals. Fig. 6B shows records from the animal in a period of the later stage of anesthesia in which rhythmic discharges of the abducens nerve tended to be replaced by tonic discharges (3). Whenever the BIN showed a high firing rate (arrow in 2) there was a strong discharge of the medial rectus nerve (arrow in 4) at the quick phase. Conversely a strong discharge of the medial rectus was usually accompanied by a high firing rate in the BIN. However the relationship was confined to the direction of nystagmus be-

cause BINs were nearly silent when the direction of nystagmus was reversed (see Fig. 7B).

### DISCUSSION

The present study has shown that the character of vestibular nystagmus is quickly affected by nitrous oxide anesthesia and that one reason for this sensitivity is that the activity of a group of premotor neurons changes soon after anesthesia onset. The burst activity of all the BINs tested was clearly changed after a short time (as soon as 10 sec) of anesthesia. Most neurons responding with a progressive decrease in firing rate and number of bursts and with an increase of burst duration. These changes were accompanied by a rounding or blunting of the end of the slow phase of the

## IMMUNODEFENCE OF THE INNER EAR?

*Lymphocyte-Macrophage Interaction in the Endolymphatic Sac*

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**Abstract** Owing to their proximity to areas exposed to infection, the sensory organs of the inner ear are probably dependent on an efficient antimicrobial defence. The longitudinal flow of endolymph to the endolymphatic sac may be of major importance in this context. Substances entering the ear can be automatically carried to the distal part of the endolymphatic duct where lymphoid cells are present and endolymphatic phagocytosis occurs. In the intermediate part of the epithelium of the sac morphological signs marking the incoming substances are present. A vigorous interaction between lymphocytes and macrophages, similar to that observed in antigen-activated lymphoid tissue, may be seen. The sac is here surrounded by a rich network of lymphatic capillaries and blood vessels.

The endolymphatic duct and sac are believed to absorb endolymph. Guild stated in 1927 that endolymph flows from the cochlear duct to the endolymphatic sac where it is absorbed and mediated to the numerous perisaccular blood vessels.

Phagocytosis by both the epithelial cells and free cells of the endolymphatic sac was demonstrated experimentally by Andersen (1948), Engström & Hjort (1950), Arnvig (1951), Lundquist (1965) and Rudert (1967).

The purpose of a phagocytic cell system inside the endolymphatic space is not known. Does the sac receive from the labyrinth effete cells or metabolic waste products too large and toxic for disposal via the cochlear duct? Or do the freely floating cells cleanse the sac of proteins normally concentrated therein and transport them via the epithelium into the surrounding blood vessels, as proposed by Ishii et al. (1966)?

Guild defined twenty-two species of cell element in the lumen of the endolymphatic

sac. These included lymphocytes whose presence prompted Linn & Silver's (1974) proposal that the endolymphatic sac acts as an immunodefensive organ for the inner ear. Since toxins or foreign substances could reach the labyrinth through the windows of the middle ear cavity it could be crucial to inner-ear homeostasis that these substances are removed and eliminated by phagocytic cells in the presence of immune cells.

In the present investigation lymphocytes and macrophages in the endolymphatic sac of guinea pigs were studied ultrastructurally. This was done in order to reveal a possible physical interaction which would indicate functional cooperation and could thus confirm the theory that the endolymphatic sac acts as an immunodefensive organ for the internal ear.

## MATERIAL AND METHODS

Fifty pigmented guinea pigs ranging in weight from 200 to 800 g were used in this investigation. The animals displayed a normal Preyer reflex. They were anaesthetized with sodium pentobarbital (Nembutal) and perfused with a solution of 2.5% (v/v) purified glutaraldehyde and 1% (v/v) formaldehyde in a solution of 0.1 M Sørensen's phosphate buffer, pH 7.2-7.4. After decapitation the middle and internal ears were examined macroscopically for signs of infection. The operculum covering the endolymphatic sac was eliminated with a fine

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nerve discharge gradually diminished and surprisingly the medial rectus nerve discharge simultaneously increased (Fig. 6A). Eventually the latter also diminished and in the deepest levels of anesthesia both nerves were silent (not shown in figure). This changing pattern could be explained as follows. After acute labyrinthectomy say on the right side type I neuronal activity in the right vestibular nucleus is much reduced because of the right labyrinthectomy and strong commissural inhibition from the left vestibular nucleus. In contrast the neuronal activity in the left vestibular nucleus is heightened because of release from commissural inhibition (Markham et al. 1977; Shimazu & Precht 1966). In this condition and under moderate anesthesia the right abducens nerve discharges tonically. This in turn is due primarily to the right abducens motoneurons receiving excitatory drive from the left vestibular nucleus (Baker et al. 1969). At the same time the medial rectus is silent as a result of decreased excitatory influence from the right vestibular nucleus and its direct projection to motoneurons (Baker & Highstein 1978).

Under deep anesthesia the right abducens nerve becomes silent and the medial rectus nerve fires tonically. The first effect may be due to the anesthetic affecting the left intact vestibular nucleus and its direct projection to the abducens nucleus while the second effect results from lessened commissural inhibition acting on the right vestibular nucleus. The reason the anesthetic acts on the vestibular nucleus on the intact side to a greater degree than on the side of the labyrinthectomy is presently unknown. However the high firing rate of vestibular nucleus neurons on the intact side versus the abnormally low firing rate on the labyrinthectomized side may be a factor.

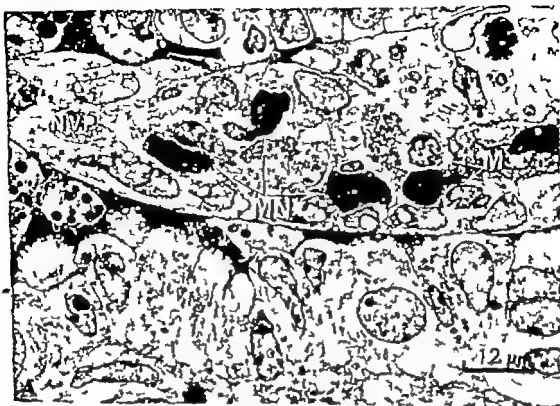
While the changes in BIN activity during anesthesia apparently account for the changes in abducens nerve discharge it must be noted that we have no experimental data explaining the concomitant changes in medial rectus discharge at the quick return phase. Through all stages of anesthesia there is a very close rela-

tionship between the firing rate of BINs and activity in the medial rectus nerve (see Fig. 6B). This means that whenever there is a particularly abrupt termination of the slow phase the ensuing quick return phase will be particularly strong.

The medial rectus nerve discharge originates from motoneurons within the medial rectus division of the oculomotor nucleus. In turn these motoneurons receive direct excitatory input from the contralateral abducens nucleus via the axons of abducens internuclear neurons (see Fig. 1 Baker & Highstein 1975). The cell bodies of these internuclear neurons are on the same side as the BINs studied here and are in close proximity to them (Highstein & Baker 1978; Baker & Highstein 1975). However since BINs have been demonstrated to be inhibitory cells some other neuron must be governing medial rectus motoneuron activity in the quick phase of nystagmus. Sensitivity of these yet unidentified "governing" neurons possibly to anesthesia (see below) to anesthesia could explain the close relationship between BIN activity and medial rectus discharge during anesthesia.

The results reported here apply only for nitrous oxide anesthesia. In two other cases we used 2% halothane with similar results although the return from anesthesia was longer than with nitrous oxide. It should be noted that the means by which drowsiness or sleep exert their effects on nystagmus may or may not be the same as for nitrous oxide.

In this study we have shown that one reason rapid eye movements are so dependent on alertness is that one set of reticular pre-motor neurons controlling abducens motoneurons is quickly affected by anesthesia. In turn one may ask why BINs are so sensitive. It is known that they receive monosynaptic input from the contralateral superior colliculus and possibly also from cells in the gigantocellular tegmental field of the ipsilateral pontine reticular formation (Hikosaka & Kawakami 1977). They also receive polysynaptic input



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Fig. 6. Lymphocytes (Ly) in the rugose epithelium of the ES. Top. The cells adhere to the epithelial cells (Ep) but display two nucleoli (Nu). Lu. Endolymphatic lumen. 2400 (left),  $\times 1750$  (right). Bottom. Pseudopodium

processes project into the cytoplasm of the epithelial cells. There are small areas of subplasmalemmal density  $\times 9800$  (left),  $\times 5400$  (right).

area of the intermediate portion of the sac were described with the help of light microscopy by Guild (1927) and ultrastructurally by Rask Andersen & Stahlé (1979). They seem to be created when freely floating cell clusters fuse with the rugose epithelium (Fig. 3). They are rich in monocytes, macrophages and lymphocytes which probably originate from the perisaccular blood vessels. The epithelial crypts trap the cell cluster while the epithelium is invaded by mononuclear cells which later

are presumably involved in the degradation of the luminal cells (Fig. 4). Lymphocyte-macrophage interaction is a common finding in these NVP (Fig. 5). The macrophages contain secondary lysosomes filled with digested material. Their large nuclei display numerous prominent nucleoli. There are broad areas of close contact between the macrophages and lymphocytes. The lymphoid cells sometimes penetrate deep into the cytoplasm of the macrophages.





Fig 1 (A) The neoplasm cords which infiltrated the larynx were surrounded by sclero-hyalin septa (G

×100) (B) The acidophilic tumour cells had a prominent nucleus and a granular cytoplasm (G ×250)

These nodes were not the metastases of an epidermoid carcinoma. After a new examination a laryngeal chemodectoma with lymphatic and subcutaneous metastases was diagnosed. Biological investigations were begun to study the functional features of this tumour; the results are discussed below as well as those of the histological and ultrastructural analyses.

In the following months no laryngeal recurrence was observed but the number of the subcutaneous nodes increased. The radiographies of the lungs, the bones, the oesogastro-intestinal tract and intravenous urography did not reveal any anomaly. The thyroid scintigraphy was normal. The patient died at home on March 25, 1975 and there was no autopsy.

## MATERIAL AND METHODS

**Light microscopy.** Tissue blocks were fixed in bouin, embedded in paraffin wax and

5 micron thick sections were cut. These sections were examined after haematoxylin-phloxin-safran, periodic acid-Schiff and Masson's trichrome stain or after Fontana and Grimelius's silver impregnations. These paraffin sections were also used for the fluorescence tests for calcitonin and carcino-embryonic antigen research.

**Electron microscopy.** In addition small specimens (1 mm<sup>3</sup>) of the dorsal nodes were immersed in 1.77% phosphate-buffered glutaraldehyde and in osmium tetroxide. The tissues were subsequently dehydrated by alcohol, embedded in araldite CY 212. Ultrathin sections were contrasted with uranyl acetate and lead citrate. The observations were made in an Hitachi 7 S or a Siemens Elmiskop 102 electron microscope.

## RESULTS

### Pathologic findings

**Light microscopy.** The laryngeal biopsy consisted of a little necrotic mass in which

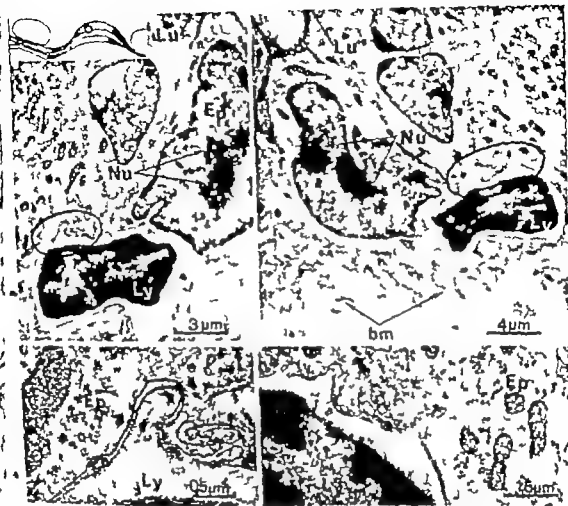


Fig. 6. Lymphocytes (Ly) in the rugose epithelium of the ES. Top. The cells adhere to the epithelial cells (Ep) each display two nucleoli (N). L: Endolymphatic lumen. 2400 (left), 1750 (right). Bottom. Pseudopodial

processes project into the cytoplasm of the epithelial cells. There are small areas of subplasmalemmal density. 5400 (left), 5400 (right).

area of the intermediate portion of the sac were described with the help of light microscopy by Guild (1977) and ultrastructurally by Rask Andersen & Stahle (1979). They seem to be created when freely floating cell clusters fuse with the rugose epithelium (Fig. 3). They are rich in monocytes, macrophages and lymphocytes which probably originate from the perisaccular blood vessels. The epithelial crypts trap the cell cluster while the epithelium is invaded by mononuclear cells which later

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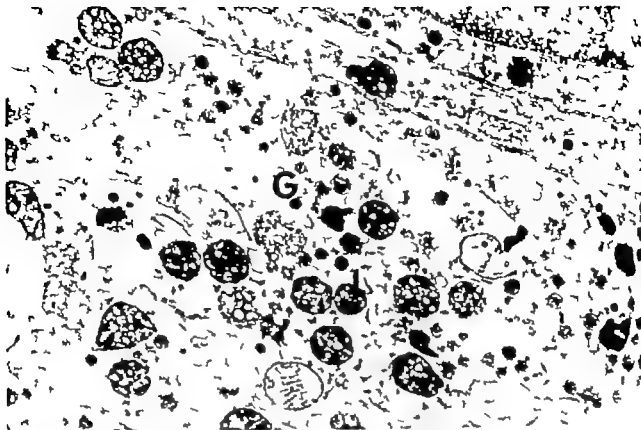


Fig 3 Several lysosomal formations (L) near by secretory granules (G), mitochondria and filaments (G  $\times 20000$ )

encircled the clusters of tumour cells. Here and there free granules were released between the stroma collagen fibers (Figs 2, 3, 4 and 5).

#### Biochemical Investigations

The TCT-like activity was more than 5 mg/ml. Blood calcitonin reached 22.87 ng/l. The fluorimetric dosage of the catecholamines



Fig 4 A few desmosomes (D) fixed the cells which were separated here and there by spaces filled up with microvilli (V) (G  $\times 6620$ ).

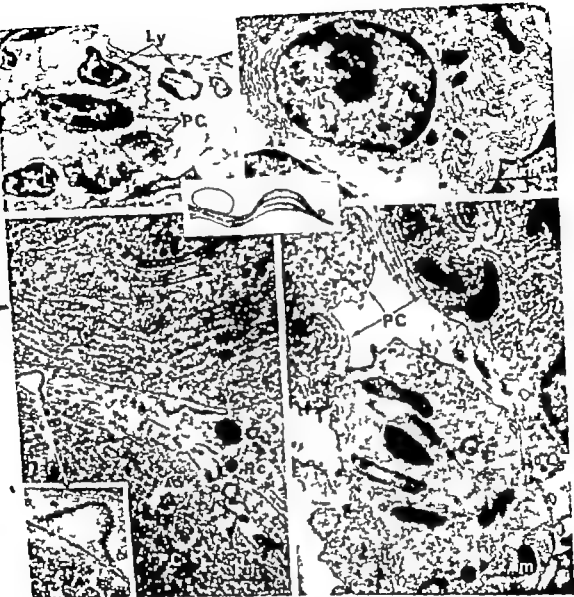


Fig. 8. Plasma cells and lymphocytes in the distal region of the sac. This region of the perisaccular tissue is so rich in plasma cells in some animals that it may be called the 'plasma-cellular' region of the ES. *Top left*: Two mature plasma cells (PC) contain large amounts of rough endoplasmic reticulum and characteristic prominent perinuclear Golgi structures. Lymphoid cells appear as 'blastoid' cells

and are scattered nearby. *Top right and bottom left*: The plasma cells (PC) have close relationship to the reticular connective tissue cells (Rc). Numerous coated pit and vesicles suggest macromolecular transport in the reticular cell cytoplasm. *5000-9400*. *Bottom right*: Plasma cells (PC) surrounded large lymphocytes. Prominent mitochondria and multivesicular body are seen. *×7500*

#### Perisaccular tissue

The intra-osseous portion of the endolymphatic sac is surrounded by an extensive network of post-capillary venules and veins. It is not un-

usual to observe small lymphocytes migrating through the thin endothelial walls into the loose tissue (Fig. 5A). Mast cells are widely distributed, many of them with pseudopods.

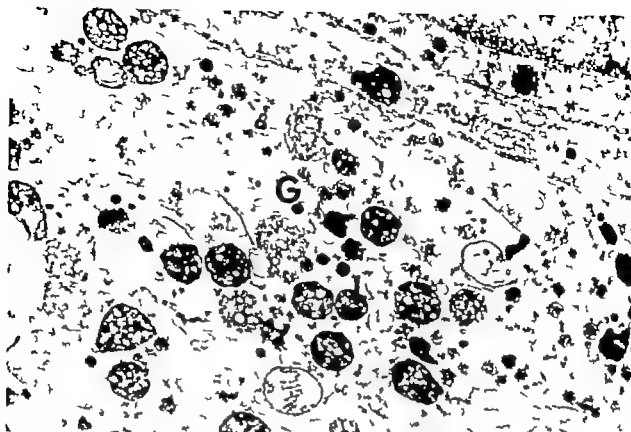


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#### *Biochemical Investigations*

The TCT like activity was more than mg/ml. Blood calcitonin reached 22.82 ng/l. The fluorimetric dosage of the catecholamine



Fig 4 A few desmosomes (D) fixed the cells which were separated here and there by spaces filled up with microvilli (V) (G  $\times 6620$ ).

## OBLITERATION OF THE DUCTUS REUNIENS

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**Abstract** The ductus reuniens was successfully obliterated in 57 guinea pig ears. Histopathological study showed that majority of these specimens demonstrated cochlear hydrops, saccular collapse and normal utricle. These results support the theory of longitudinal flow of endolymph from the cochlea toward the endolymphatic sac via the ductus reuniens and sacculus. A major source of endolymph in the sacculus appears to be the scala media. In another set of 11 animals in which the ductus reuniens was first obstructed and two months later the endolymphatic duct was blocked, endolymphatic hydrops as shown in the cochleae, saccules, and utricles of all but one. The evidence suggests that cochlear hydrops was caused by obliteration of the ductus reuniens, and the saccular and utricular hydrops occurred subsequently as the result of blockage of the endolymphatic duct. Remnants of otolithic membrane which were attached to the distended saccular wall indicate that the membrane which had collapsed onto the sacculus after obliteration of the ductus reuniens is capable of subsequent distension. This experiment supports the concept of endolymph flow from the utricle and canals toward the endolymphatic sac. A blocked ductus reuniens might also explain the pathophysiological basis for the sensory form of Meniere's disease.

There are two principal theories concerning endolymph secretion and absorption in the membranous labyrinth. In the longitudinal flow theory, endolymph of the cochlea is produced in the scala media and passes through the ductus reuniens, saccule and endolymphatic duct to reach the endolymphatic sac where absorption takes place (Guild 1927, Lundquist 1965). Guild (1927) postulated that in the vestibular system there is a flow of endolymph from the utricle and ampullae through the utriculo-endolymphatic valve toward the endolymphatic sac. The radial flow theory contends that endolymph is both produced and absorbed locally within the scala media (Naftalin & Harrison, 1958; Lawrence & Mc

Cabe 1959; Lawrence et al. 1961; Lawrence 1966) as well as in the utricle (Dohlman 1964, 1965). The radial flow theory appeared to gain support from reports by Lindsay (1947) and Suh & Cody (1977) that no change in membrane position occurred after obliteration of the endolymphatic sac in the monkey and chinchilla respectively. On the other hand, the occurrence of consistent endolymphatic hydrops in other species of mammals after obliteration of the endolymphatic duct or sac (Kimura & Schuknecht 1965; Kimura 1967, 1976; Schuknecht et al. 1968; Beal 1968 and others) supports the longitudinal flow theory.

Since the results of these experiments are clearly in conflict, an experiment was designed to further test the validity of the radial or longitudinal flow theory in the guinea pig which is ideally suited for this type of experiment. The experiment consisted of morphological studies on two groups of guinea pigs: one with obstruction of the ductus reuniens alone and the other with obstruction of the ductus reuniens followed later by obstruction of the endolymphatic duct. The findings might also have relevance concerning individual dependence of the auditory and vestibular labyrinths on the absorptive function of the endolymphatic sac. Furthermore, the experiment might shed light on the pathophysiology of atypical forms of Meniere's disease. It is known that endolymphatic hydrops is the principal pathological feature of this disease and that most affected patients will exhibit both auditory and vestibular symptoms. Atypical cases do occur however, where the symptoms and functional test

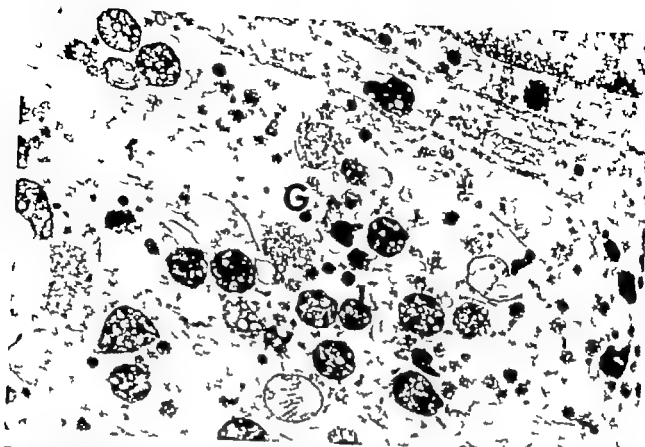


Fig 3 Several lysosomal formations (L) near by secretory granules (G) mitochondria and filaments (G  $\times 20000$ )

encircled the clusters of tumour cells. Here and there free granules were released between the stroma collagen fibers (Figs 2, 3, 4 and 5).

#### Biochemical investigations

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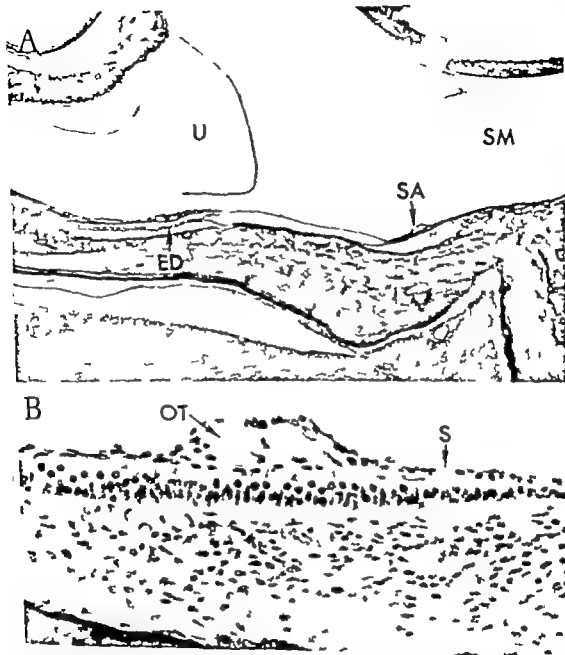


Fig. 3. The same specimen as shown in Figs. 1B and 2A. (A) The saccular membrane is collapsed after ductus reuniens obliteration. The distended scala media (SM) is shown at the right. The endolymphatic duct (ED) is

normal and patent. SA: Saccule- U: utricle. (B) A higher magnification of the macula sacculi in Fig. 3A showing the degenerated otolithic membrane (OT). The mass is surrounded by the saccular membrane (S).

As controls of the experiment, the round windows were opened in six ears, the cochlear ducts were perforated through both Reissner's and basilar membranes in 13 ears and sta-

pides were mobilized or subluxated in seven ears. The saccular perforations performed in nine ears of an earlier experiment (Kimura et al. 1977) served as another experimental con-



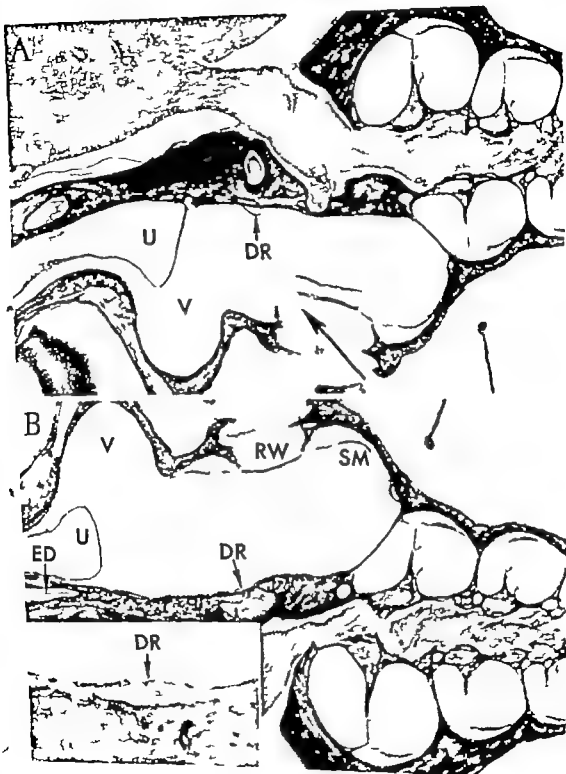
Table I *The laryngeal chemodectomas*

| No | Year | Author                | Sex | Age | Evolution   |
|----|------|-----------------------|-----|-----|---|
| 1  | 1915 | Andrews               | M   | 77  | No recurrence   |
| 2  | 1955 | Blanchard & Saunders  | M   | 38  | No recurrence   |
| 3  | 1955 | Zeithofer             | F   | 56  | No recurrence   |
| 4  | 1958 | Schall                | F   | 49  | ?   |
| 5  | 1960 | Hartmann              | F   | 40  |   |
| 6  | 1962 | De Barros             | F   | 44  | ?   |
| 7  | 1965 | Wockel et al          | F   | 56  | No recurrence   |
| 8  | 1965 | Wockel et al          | M   | 46  | Local recurrence  |
| 9  | 1965 | Baxter                | F   | 59  | No recurrence   |
| 10 | 1965 | Gignoux et al         | F   | 21  | No recurrence   |
| 11 | 1966 | Orty                  | ?   |     |   |
| 12 | 1967 | Martinson             | M   | 14  | No recurrence   |
| 13 | 1968 | Azevedo-Gamas & Gloor | F   | 36  | No recurrence   |
| 14 | 1970 | Vetters & Toner       | M   | 60  | No recurrence after resection of the tumour and of a lymphatic metastase    |
| 15 | 1971 | Benevise et al        | F   | 30  | No recurrence   |
| 16 | 1971 | Ishida et al          | M   | 19  | ?   |
| 17 | 1972 | Adlington & Woodhouse | M   | 51  | Dissemination with lymphatic and subcutaneous metastases                    |
| 18 | 1972 | Hooper                | M   | 55  | Dissemination with lymphatic and subcutaneous metastases                    |
| 19 | 1972 | Hooper                | M   | 64  | Dissemination with lymphatic and subcutaneous metastases                    |
| 20 | 1972 | Hooper                | M   | 54  | No recurrence   |
| 21 | 1972 | Laudadio              | F   | 45  | No recurrence   |
| 22 | 1972 | Tobin & Harris        | M   | 67  | No recurrence   |
| 23 | 1972 | Zachariah & Shah      | M   | 61  | Local recurrence and general dissemination                                  |
| 24 | 1975 | Greenway              | F   | 46  | No recurrence   |
| 25 | 1976 | Fraldoon              | M   | 54  | No recurrence after radiotherapy of the tumour and of a lymphatic metastase |
| 26 | 1976 | Markowska et al       | F   | 42  | No recurrence   |
| 27 | 1976 | Piquet et al          | F   | 51  | No recurrence   |
| 28 | 1977 | Lack et al            | F   | 57  | No recurrence   |
| 29 | 1978 | Hohbach & Mootz       | M   | 61  | No recurrence   |
| 30 | 1978 | Perris et al          | F   | 41  | No recurrence   |
| 31 | 1979 | Justrabo et al        | M   | 53  | Dissemination with lymphatic and subcutaneous metastases                    |

larger the inferior glom found between the cricoid cartilage and the first tracheal ring were less affected. All these bodies are composed of polygonal epithelial like cells surrounded by a vascular connective tissue (Fig 6).

This assertion is confirmed by the following facts. The right aryepiglottic fold was destroyed in nine cases and the left one in three patients. In seven other cases the left aryepiglottic fold, the false cord, the pyriform fossa, the ventricle and the true cord were simultaneously colonized. Three tumours infiltrated the right aryepiglottic fold, the right false cord and the ventricle. One was located to the right pyriform fossa and

invaded the homolateral true cord, two were subglottic and two supraglottic. The location in the four other cases was unknown. For several months or years before the first examination only a hoarseness, cervical pain and a dysphagia had been noticed in all the observations. The indirect laryngoscopy showed a submucosal mass whose biopsy was often difficult, haemorrhagic and unsuccessful. Then a laryngeal epiglottectomy or a tumorectomy were performed. The neoplasm 1.5 to 2 cm in diameter could reach 6x4 cm (Andrews 1915, Tobin & Harris 1972) or was as small as rice-grain (De Barros 1967). It was limited by a capsule with a well developed venous



ables ont déjà été rapportées. Les glomus laryngés supérieurs et plus accessoirement les glomus inférieurs sont le siège de ces tumeurs qui surviennent avec une égale fréquence dans les deux sexes. Un enrouement est souvent le seul symptôme signalé pendant les mois voire les années précédant le premier examen de cette tumeur à croissance lente. Néanmoins les chemodectomes laryngés se différencient des autres chemodectomes de la tête et du cou par un plus mauvais pronostic. Cinq des malades sont décédés dans un tableau de dissémination métastatique deux étaient porteurs de métastases ganglionnaires lymphatiques et un autre a présenté une récurrence locale. Les caractères ultrastructuraux et parfois l'activité fonctionnelle incontestable de ces chemodectomes sont ceux d'apudomes dont les cellules dériveraient d'éléments ayant migré de la portion céphalique des crêtes neurales jusqu'au larynx.

## ZUSAMMENFASSUNG

Die Autoren berichten über einen Fall von Chemodectoma laryngum bei einem 53-jährigen Mann. Gestorben mit genereller Dissemination sowohl wie mit lymphatischen und unterepidermischen Metastasen. Dieser funktionierende Neoplasma produzierte große Mengen von Calcitonin, bestimmt durch biochemische Untersuchungen und bei Immunofluoreszenz. Vielleicht produzierte er auch adrenalinähnliche Substanzen. Seine Zellen die den Hauptzellen des Glomus caroticum gleichen waren mit neurosekretorischen Körnern und Lysosomen gefüllt. Dreißig gleiche Fälle sind schon beschrieben worden. Diese Tumoren des Glomus caroticum vorkommen in dem oberen Glomus laryngum und seltener in dem niederen anzutreffen. Heiserkeit ist gewöhnlich während Monate sogar Jahre das einzige Symptom dieser langsamwachsenden Tumoren. Jedoch unterscheiden sich die laryngischen Chemodectome von den anderen Kopf- oder Hals-Chemodectomen bei einer schlechteren Prognose. Fünf Patienten sind mit einem Bild von metastatischer Dissemination gestorben, zwei trugen Lymphknotenmetastasen und ein Patient hatte lokale Rezidive. Die Ultrastruktur sowie die zweifelhafte funktionale Aktivität dieser Chemodectome gleichen den Apudomen in welchen die Zellen wahrscheinlich von der cephalischen Neuralleiste abstammen.

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In the series of 11 animals in which the endolymphatic duct was blocked two months after surgical obliteration of the ductus reuniens through the round window hydrops was observed in all cochlear sacculi and in 10 utricles (Fig. 5). The magnitude of distension of these membranes was similar to that which follows blockage of the endolymphatic duct alone. In 8 of 11 ears the distended walls of the sacculi contained small remnants of the atrophic otolithic membranes structures similar to those observed on the collapsed saccular maculae in the series undergoing blockage of the ductus reuniens (Fig. 6). Other histopathological changes were neurosensory lesions at the basal ends or in both basal and apical turns similar to those seen in the ductus reuniens series. Two specimens showed sensory cell atrophy in small areas of the saccular maculae. The sensory cells of the utricular maculae and cristae were normal except for three posterior cristae which showed degeneration.

### DISCUSSION

It might be argued that all these pathological alterations could be the result of a non-bacterial traumatic labyrinthitis and therefore are not related to obliteration of the ductus reuniens. Of course all invasive surgical procedures produce inflammatory and reparative responses. One method of experimental control is to perform another invasive procedure which is similar but selectively different from the one under study. When such controls were performed (opening of the round window perforation of the scala media, stapes mobilization) the resultant histopathological features

were significantly different from those following obliteration of the ductus reuniens. Even in our simple sacculotomy series (Kimura et al 1977) results were unlike those of the present ductus reuniens series. Thus histopathologic observations in the majority of the specimens appear to be related to obliteration of the ductus reuniens. Various other histopathological features in a small number of specimens could be attributed to labyrinthitis: specimen preparation artifact tears in other parts of the membranous wall or simply non-healing of the fistulized membranes.

The main histological characteristic after obliteration of the ductus reuniens was the combination of cochlear hydrops, saccular collapse and normal utricle. The combination with a much lower incidence was cochlear hydrops and normal sacculus and utricle. The cochlear hydrops may be explained if we presume that endolymph normally flows toward the ductus reuniens sacculus and endolymphatic sac. If the ductus reuniens were blocked and endolymph were still being produced then Reissner's membrane would distend. Supporting evidence from another experiment is the demonstration of cochlear hydrops after blockage of the endolymphatic duct, as stated earlier. In a labyrinthotomy experiment in which the hook region of the cochlea was surgically destroyed the remaining parts of the cochlea developed hydrops (Hoshino & Paparella, 1970). Gussen (1978) reported that atrophy of the human macula sacculi resulted in accumulation of otoliths or otolithic membrane debris in the ductus reuniens and/or in the cochlear duct. The tissue reaction at these areas caused development of hydrops in the basal turn and even throughout the cochlea.

The sacculus in the present study collapsed possibly because it was deprived of its main source of endolymph from the scala media. There are no secretory cells (dark cells) in the sacculus (Kimura, 1969). A close physiological relationship between the cochlea and sacculus was also shown by the collapsed Reissner's

Fig. 6. The saccular membranes of specimens in which the ductus reuniens was obliterated first followed by blockage of the endolymphatic duct two months later. (A) The saccular membrane with the remnant of the otolithic membrane (OT) is partly lifted as the hydropic sacculus (SA). ST Stapes footplate. (B) The remnant of the otolithic membrane is attached to the distended saccular membrane. The same specimen as shown in Fig. 5C. EN Endolymph side. (C) Another specimen showing the lifted degenerated otolithic membrane attached to the distended saccular membrane. EN Endolymph side.

## DYSPRAXIA OF SPEECH AND OF EYE MOTILITY

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**Abstract** Five patients with a disturbance of their preprogramming of speech (dyspraxia of speech) were exposed to a comprehensive eye-motor test-battery. The saccades were found hypometric and inaccurate with irregular intervening pauses. The finding was interpreted as due to an extension of lesions from frontal cortical speech areas into visual motor cortex disturbing the normal preprogramming of voluntary saccades. The results were interpreted as supporting the assumption that voluntary saccades are initiated in the frontal cortex. An increase of vestibular nystagmus in these cases was related to a release of vestibular nystagmus due to the disappearance of cortical inhibition on brain stem activity.

This paper will deal with disturbances in the same patients of two seemingly unrelated voluntary activities that of preprogramming of speech and that of preprogramming of voluntary fast eye movements: the voluntary saccades. Both types of activities demand the will of the patient as well as a large amount of coordination of muscle activity and both activities may be triggered in the frontal cortex in front of the pre-central gyrus.

This specific disturbance of programming of speech may be due to a lesion in the frontal cortex resulting in a typical speech pattern (Darley et al 1975). In these cases although the persons are capable of finding the right word from their "bank of words" they are unable to initiate a proper combination of muscle activity to produce the word. Instead they will produce with a normal tone and articulation and normal strength faulty constructions of that word. They are aware of their mistakes and will repeatedly try to

correct themselves frequently without success.

This disturbance of speech has been called apraxia (Ljepman 1914 Darley et al 1974). Since this term would indicate a complete inability to control speech which rarely is the case we have suggested for this disturbance and have in this paper used the term dyspraxia.

In these patients a pronounced decrease of accuracy of fast eye movements was frequently found. These patients seem to have lost their normal ability to preprogram voluntary eye movements together with their ability to preprogram speech. This combination initiated the present study of various types of eye movements in patients in whom the dyspraxia of speech indicated a frontal cortical lesion.

### MATERIAL AND METHODS

Five patients with a speech disorder of the dyspraxic type were examined and compared with five age matched normal subjects (Table I). The speech disorder was classified according to conventional phoniatric tests (Johns & Darley 1970). Great care was taken to exclude patients with impulsive or expressive aphasia from the study. However in all patients with dyspraxia a component of expressive aphasia could be detected. This usually was of a minor degree when compared with the disorder caused by motor dyspraxia.

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## DISCUSSION

*Wersäll to Kimura* I have always admired Dr Kimura's experimental surgical ability. I want to congratulate you on the quality and consistency of your studies. I find your present work very important for the understanding of endolymph circulation. I have only one question. We know now that the dark cells are only found in the areas in the vestibular part of the labyrinth where we have endolymph production. Can you speculate on the function of the dark cells?

*Spoendlin to Kimura* What is the size or rapidity of endolymph flow toward the endolymphatic sac? The second question is what is the surgical approach to the ductus reuniens through the otic capsule?

*Saidi to Kimura* Your excellent experiments leave little doubt that there is an endolymphatic flow from the cochlea to the endolymphatic duct and sac, and that blocking of this pathway results in distention (hydrops) proximal to the obstruction. However as a surgeon I must ask myself as to the functional meaning of the endolymphatic duct (or sac) obstruction. Because I (like other ear surgeons) have seen and published cases where slow bone resorption associated with deep cholesteatoma has destroyed the entire labyrinth—at times not sparing the vestibule wall (sparing only the cochlea), while the hearing before and after the operation showed normal or near-normal bone conduction. In these ears the endolymphatic duct, as obviously included in the destructive process. If such ears developed histological endolymphatic distention—the so-called

'hydrops'—then this hydrop caused them little harm. This tallies all with the observation that such endolymphatic distention as found in Meniere patients is also found in multitude of other ear diseases or indeed at times in ears with no history of deafness or vertigo.

It would therefore seem that narrowing or closing of the endolymphatic duct and sac might be less dangerous than is usually assumed—and that the histological picture of a dilated endolymphatic system is unspecific and possibly does not always signify pathological process.

The connotations of the above to the various treatments applied to patients suffering from vertigo are self-evident.

*Thalmann to Kimura* I am particularly impressed by the collapse of the saccule since this is further proof of the secretory role of the dark cells which according to your previous studies are lacking in the saccule. Also the resting potential of the saccule has been shown to be merely remote manifestation of the endocochlear potential. Your experimental pathological results agree heartily with current electrophysiological concepts about the role of the saccule.

## Kimura (Reply)

*T. Wersäll* I am very grateful for your nice comments. At present I cannot give you more information on the function of dark cells. I am sure that future study will reveal more specific function of the dark cells.

*T. Spoendlin* It is my impression that the size of the endolymph flow toward the endolymphatic sac is rather small and slow. This opinion is based on my experience of opening the normal endolymphatic sac. However it should be noted that hydrops develops in the cochlea and saccule within 24 hours after obliteration of the endolymphatic duct. The surgical approach to the ductus reuniens through the vestibule is to open the bony wall posterior to the round window. This is accomplished by thinning the bone with a burr and deep circular groove is made with a pointed instrument. The bony disc and fragments are lifted out with a fine hook. A long curved needle is inserted to cut the ductus reuniens of which the location was predetermined by the previous anatomical study.

*T. Saidi* I do not have an answer to your clinical observations. The histopathological study related to the present investigation is a case of hydrops limited to the cochlea without having the typical symptoms of Meniere's disease as reported by Lindsay and von Schultze (1958). Olsson reported last year that cochlear hydrops was noted in the cases of cochlear and saccular degeneration. She attributed hydrops to the degenerated otolithic membrane entering into the ductus reuniens and the recess vestibular where fibrosis and inflammatory reaction developed.

*T. Thalmann* Collapse of the saccule membrane is a real phenomenon. I am still convinced that there are no dark cells in the saccule. The main source of endolymph is probably the scala media. Distribution of dark cells in the vestibular labyrinth is mapped in my previous publication. They are shown in all three ampullae and utricle. A part of endolymph in the saccule may come from dark cells of the utricle and ampullae.

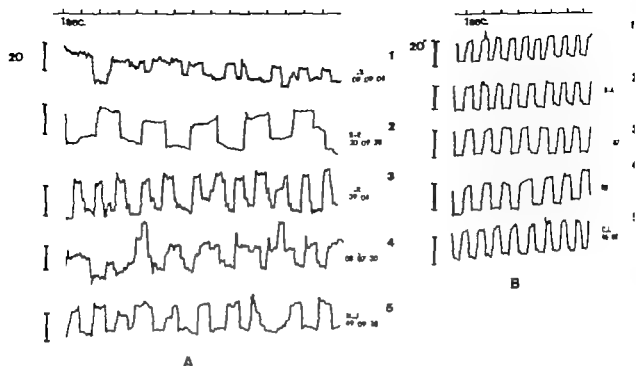


Fig 1 The command eye movements of 20° (A) in patients with dyspraxia (B) in healthy controls. The

time scale is shown in the uppermost curve in pictures

patients 3, 4 and 1. The same patients showed a disturbance of following eye movements. When comparing the optokinetic patterns of normals and of the diseased there were no clear-cut differences. However the amplitude of optokinetic nystagmus of the patients was slightly larger than that of the normals.

#### 4 The eye movements in rotation tests

Vestibular nystagmus induced by rotation in darkness was normal in all patients except no. 4 in whom the amplitudes were very low. The nystagmus was rhythmic especially so in patients 1 and 5 (Fig 4). The average amplitude was larger in the group of patients than in the group of normals.

#### 5 Eye movements in caloric tests

The nystagmus caused by caloric irrigation of the ears in the dark was remarkably regular and quite symmetrical in all patients (Fig 5). The amplitudes of the normals were definitely smaller than those of the patients. A nystagmus of remarkably high amplitude was found with in patients 1, 3 and 4. These

patients showed a disturbance of the following movements and a lower amplitude optokinetic nystagmus than the two remaining patients.

In an effort to evaluate the correlation between the different nystagmus responses the pursuit ability four characters ranked for comparison: the smoothness of the following movements and the amplitude of caloric optokinetic and postrotatory nystagmus. In this way the amplitude of caloric and of optokinetic nystagmus could be plotted against smooth tracking ability (Fig 6). This plotting indicates that caloric amplitudes decrease with increasing tracking ability while optokinetic nystagmus increases with this ability. The postrotatory nystagmus pattern was so similar in four cases that ranking could be made.

## RESULTS

Patients with dyspraxia of speech produced saccades of normal velocity but with pronounced dysmetria and with prolonged pauses between saccades. In spite of this

Unseren Befunden nach ist sie ein Teil der herdförmigen perilymphatischen Fibrosierung die beherrschendes morphologisches Substrat dieser Erkrankung ist

4 Die Veränderungen an der Stria vascularis möchten wir nicht wie Wolff und Mitarbeiter als Hauptursache des Hydrops anführen. Sie könnten sicher beitragen durch vermehrte Lymphproduktion was aber bis heute nicht beweisbar ist

Unsere morphologischen Befunde erlauben aber auch Rückschlüsse auf die Ätiologie der Erkrankung. Da unser Fall allerdings kein akuter war können wir zu den sehr bemerkenswerten Befunden erhoben von Edström und Vahlne nicht Stellung nehmen. Wenn wir allerdings die Beobachtungen an den Gefäßen im Felsenbein als panarteritis-nodosa ähnlich festlegen so wird die Störung im Immunsystem, über welche sie als Infektionsfolge berichten sehr wichtig. Auch unser Patient erlitt den ersten Schub des Hörverlustes im Rahmen einer Infektion. Ob und wie eine Autoimmunerkrankung daraus entstanden ist können wir mangels Befunden an anderen Organen nicht festlegen. Aber die Veränderungen an den Gefäßen rund um den Nervus facialis und an der Arteria labyrinthi sowie die Thrombosen in den Venen der Kogangswand und der Begleitvene des Aqueductus vestibuli können als pathologische Beweis gelten. Wir möchten deshalb Olmer und Mitarbeiter sowie Fisher und Hellström unterstützen und auch Cogan und Dickerson, die annehmen daß das Cogan Syndrom eine Teilform der Panarteritis nodosa ist, ähnlich der Wegener'schen Granulomatose in unserem Fachgebiet. Somit wäre das Syndrom den Autoimmunerkrankungen zuzurechnen, wofür auch die Therapieerfolge mit hochdosiertem Cortison berichtet von Smith 1970 sprechen würden.

## ZUSAMMENFASSUNG

Es wird über pathologische Befunde erhoben an einem menschlichen Felsenbein beim Cogan Syndrom berichtet. Die bemerkenswerten Beobachtungen sind: 1

Fibrose im perilymphatischen Raum Scala vestibuli systema perilymphaticum vestibuli rund um den oberen Bogengang und den Ductus rotundus. 2 Ossifikationen im perilymphatisch-fibrosierten Annulus: Scala vestibuli der basalen Schneckenwindung. Vestibulum. 3 Zerstörung der Störungsstellen: Corti'sches Organ macula sacculi und utriculi. 4 Endolymphatischer Hydrops mittlere, obere Schneckenwindung, Sacculus, Utriculus oberer Bogengang. 5 Perisacculäre Fibrose im Ductus endolymphaticus und Blockade der Basal'schen Klappe. 6 Panarteritis nodosa ähnliche Gefäßveränderungen an der Arteria labyrinthi den Venen um den Aqueductus vestibuli und an allen Gefäßen um den Nervus facialis. Auf Grund dieser Veränderungen nehmen wir an daß es sich beim Cogan Syndrom um eine lokalisierte Erkrankung aus dem Formenkreis der Autoimmunerkrankungen handelt.

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## DISCUSSION

Kellemen to Zechner: Records of birth of Cogan syndrome at the Massachusetts Eye & Ear Infirmary until its knowledge grew as shown in the masterful presentation.



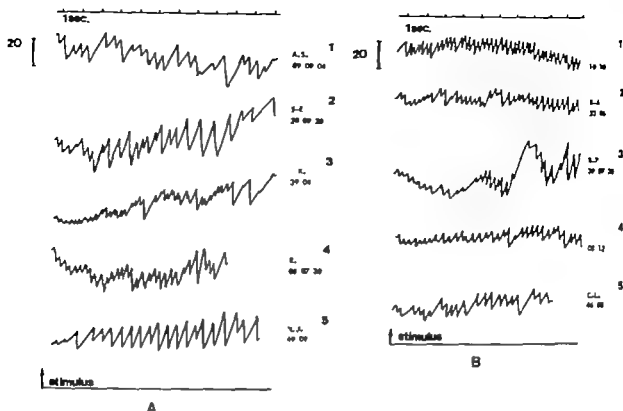


Fig 3 The optokinetic eye movements (A) in patients with dyspraxia, (B) in healthy controls

normal force. The corresponding dimension in eye movement should be the velocity. The velocities of the disturbed saccades were accordingly found normal in spite of the splitting up into many saccades.

**C Tempo.** A certain prolongation between consecutive letters, syllables or words seems typical of the dyspractic speech. The tempo is staccato with long intermissions during which the patient seems to search for the appropriate word or syllable. In eye movements this pattern is reflected by prolongations of or variations in the periods between consecutive saccades.

We may now claim at least that these three features of speech, accuracy, strength and tempo, are reflected in corresponding features in patterns of eye movements.

## 2 Location of excitatory neurons for voluntary saccades

Earlier concepts about the role of cortex initiating eye movements were largely based

on clinical observations (Holmes 1953; Cogan & Adams 1953) ablation studies (Laito & Cowey 1971; Pasik & Pasik, 1971) and observations of eye movements induced by stimulation of the cortex (Ferner 1937; Penfield & Boldrey 1937; Pasik & Pasik 1964). All these studies indicated that voluntary saccades are probably triggered by a part of the frontal cortex. This assumption was further supported by observations that radiologically positive premotor potentials have been shown in EEG in the frontal and parietal areas (Becker et al 1968; Barlow & Colquhoun 1969; Kurtzberg & Vaughan 1973).

Recent experiments with single unit recordings have however largely contradicted these concepts. Thus in single unit recordings Bizzzi (1968), Bizzzi & Schiller (1971) and Mohler et al (1973) were not able to detect any presaccadic activity in the prefrontal area although they could record activity during the saccade as well as during slow eye movements.



Figs 3-11 EM aspects of damaged structures in the basal coil of pup cochlea 7 days after transtibial exposure

Figs 3-4 Apical pole of OHCs. Note numerous lysosomes (L), vacuoles (V) and Golgi structures (G) 16000

Figs 5-6 IHC showing the same cytoplasmic inclusions as in OHC. Note on Fig 5 damaged stereocilia (arrow) 10000 (5), 17000 (6).

Figs 7-8 Affluent fibres spiralling between Deiter's cells with vacuoles and myelin figures (MF). 16000 (7), 9000 (8)

Figs 9-10 Efferent endings to OHC. Note the abnormally low density of synaptic vesicles and myelin figures (MF). 15000 (9), 16000 (10)

Fig 11 Myelin figure complex (Sponadlin 1970) in supporting IHC 13000

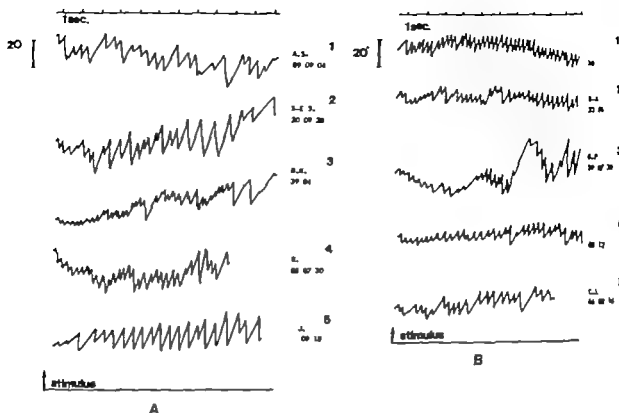


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## INVESTIGATIONS ON THE CRITICAL PERILYMPHATIC PRESSURE VALUE CAUSING ROUND WINDOW MEMBRANE RUPTURE IN ANESTHETIZED CATS

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**Abstract** Increasing perilymphatic fluid pressure was found to be an important factor in the etiology of round window membrane rupture. The critical pressure causing membrane rupture was determined in anesthetized cats. Its value was found to be in the range 10-30 mmHg ( $23.5 \pm 8$  E.M.L. =  $23.4 \pm 17.1$ ). This value was compared with the pressure increases caused by thoracic and abdominal compression, cervical strangulation, forced Trendelenburg position, coughing and sneezing.

Although pressure and flow conditions in the perilymphatic space have been investigated from several aspects in the last couple of years, one can find scarcely any reference in the literature about the critical perilymphatic pressure values causing rupture of the round window membrane.

In the present series of experiments—carried out in anesthetized cats—we have investigated this critical range of the perilymphatic pressure.

The pressure values causing rupture of the round window membrane were compared with those increased perilymphatic pressure values which could be measured in different, experimentally induced physiologic and pathophysiologic conditions in the same experimental animals.

### METHODS

The experiments were performed on 12 female cats weighing 2½–3½ kg and anesthetized with 40 mg/kg sodium pentobarbital (Nembutal, Abbott, Ottignies Belgium) intravenously.

The trachea was cannulated with a glass

cannula, and the bullae osseae were prepared free on both sides from incisions led behind the ears. The round window was exposed by opening the bulla. In one group of the animals (6 cats) a 1.5 mm hole was opened with a dental drill to the perilymphatic space through the bony wall of the round window at some millimetres distance from the window. In another group of animals (6 cats) the perilymphatic space was opened by stapedectomy.

A 1.0 mm outer diameter metal cannula was inserted into the hole and fixed with Duracryl (Duracryl "O" self-curing resin SPOFA-Dental Prague) dental cement. The cannula was connected to a short polyethylene tube, filled with Evans-blue stained physiological saline. The tube was connected to a three-way stopcock, the first branch of which was connected to a mercury manometer whose pressure values were recorded on a kymograph. The second branch was connected to an electric pressure transducer (P 23 AA Statam, Elm Inc., Puerto Rico, USA) in order to have a simultaneous possibility for recording low "break-through" pressure values on a polygraph (C.F. Scime Galileo R 105 h, O.T.E. Biomedica, Florence, Italy). The pressure in the perilymphatic space was raised slowly and continuously through the third branch of the stopcock with the aid of a Mantoux syringe filled with Evans-blue stained physiological saline.

The experiments were carried out only in those animals in which the round window membrane was intact under the control of an operating microscope ( $\times 37$  magnification).

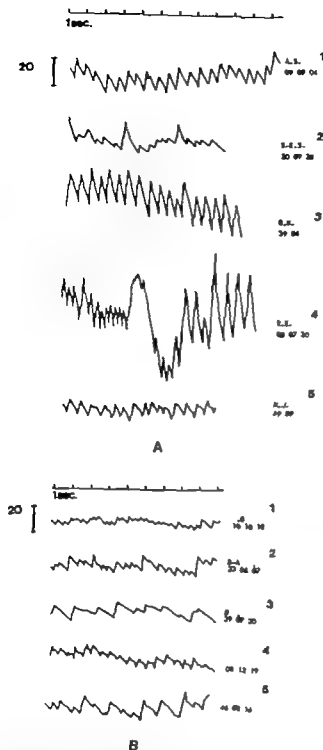


Fig 5 Nystagmus recorded after cold water (30°) irrigation of right ear: (A) in patients with dyspraxia (B) in healthy controls.

in man. It may be suggested that most of the eye movements studied in monkeys in laboratories were initiated by interest and the eye movements thus excited in area 7 (Lynch et al 1977). The command eye movements in man may be an effect of a

Ranked by target amplitude

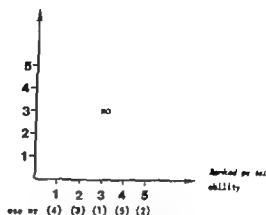


Fig 6 Ranked amplitude of caloric (x) and of optokinetic (o) nystagmus as a function of pursuit ability

process in frontal cortex: phylogenetically newly developed speech

### 3 Increase of caloric nystagmus amplitude—an effect of reduced cortical inhibition?

An intense and rhythmic caloric nystagmus response was found in all dyspraxic subjects. The largest amplitudes were found when disturbances of saccades as well as pursuit eye movements indicated more extensive cortical lesions. The large nystagmus amplitude may thus be an effect of reduction of cortical inhibition of a meaningless vestibular response.

A careful analysis also of properties of the saccades may then be useful for evaluation of the vestibular responses and also helpful for discriminating between patients with aphasia and dyspraxia which is of interest for the phoniatrist and his selection of treatment.

### ZUSAMMENFASSUNG

Fünf Patienten mit Sprechprogrammierungsstörungen (oraler verbaler Dyspraxie) wurden mit einer umfassenden Serie von Prüfungen der Augenmotorik untersucht. Es ergaben sich hypometrische und ungenauere Saccaden mit unregelmäßigen zwischenliegenden Pausen. Die Befunde deuten auf eine Ausbreitung der kortikalen Läsionen vom frontalen Abschnitt für Sprache bis zum motorischen Cortex für Augenmotorik mit daraus resultierenden Störungen der Vorprogrammierung von willkürlichen Saccaden. Diese Resultate bestätigen die

Table II Maximum perilymphatic pressure increases (in mmHg) under the influence of test situations

| Type of the test               | Cervical strangulation | Thoracic compression | Forced Trendelenburg position | Coughing | Sneezing |
|--------------------------------|------------------------|----------------------|-------------------------------|----------|----------|
| Number of experimental animals | 3 cats                 | 7 cats               | 5 cats                        | 5 cats   | 5 cats   |
| Inner ears                     | 3 ears                 | 13 ears              | 10 ears                       | 7 ears   | 5 ears   |
| Measurements                   | 9 cases                | 24 cases             | 22 cases                      | 36 cases | 29 cases |
| $\bar{x}$                      | 1.09                   | 0.75                 | 1.79                          | 1.14     | 1.18     |
| S.E.M.                         | 0.31                   | 0.11                 | 0.34                          | 0.12     | 0.15     |
| S.D. ±                         | 0.93                   | 0.53                 | 1.58                          | 0.72     | 0.80     |

ture changes are shown on Table II. As can be seen in this table, the most significant pressure increase was observed during the forced Trendelenburg position. In some of these cases we have observed a pressure increase of 5.8 and 4.0 mmHg magnitude, which values are close to the lowest pressure values causing round window membrane rupture in our experiments.

### DISCUSSION

The symptoms of the round window membrane rupture—according to the literature data—can be observed in the form of serious disturbances of the cochlear and vestibular function and ear noises (Summons 1968, Goodhill 1971, 1973, Pullen 1977, Freeman et al. 1974, Healy et al. 1974, Taylor & Bricknell, 1976, Allam 1976, Althaus 1977, Goodman & Morooka, 1978, Kleinfeldt 1978, Mohr et al. 1978, Behbehani & Kastenbauer 1978).

Experimental studies published in the last couple of years have provided a considerable amount of data on the pathophysiological background of the dysfunctions (Summons et al. 1962, Arnold & Ilberg 1972, Lamkin et al., 1975, Axelsson et al. 1977, Ivarsson & Pederlen, 1977, Weiskopf et al. 1978).

The data of Summers (1962, 1968) and Goodhill (1971, 1973) have called attention to the significance of the perilymphatic pressure changes in the development of perilymphatic

fistulas. The aim of our present investigations was to prove the connection between the perilymphatic pressure increase with the rupture of the round window membrane in experimental conditions and also to determine the pressure range of the perilymphatic fluid in which round window membrane rupture can occur. The results of the animal experiments presented show that increased perilymphatic pressure can lead definitely to rupture of the round window membrane. The average of the critical pressure values was between 20 and 30 mmHg. The highest and the lowest pressure values at which membrane rupture was observed in the experiments were 66 and 6 mmHg, respectively. In this pressure range there was no accompanying oval window membrane rupture. This fact makes it certain that the tissue structure of the oval window membrane is more resistant to the increased pressure than is that of the round window membrane in spite of the observation that the tissue layer covering the perilymphatic space from inside appears to be a homogenous anachnoid structure (Frank 1979).

According to our present results, the pressure values causing rupture of the round window membrane can show considerable individual differences and significant differences were found in this respect even in the two inner ears of the same animal. We assume that this phenomenon is probably due to the structural and mechanical resistance differences of the membranes. The perilymphatic pressure

# THE ORIGIN OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) NERVES IN THE FELINE NASAL MUCOSA

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(Received April 4 1979)

**Abstract** The feline nasal mucosa is richly supplied with vasoactive intestinal polypeptide (VIP) immunoreactive nerve fibres. The nerves occur in the subepithelial connective tissue around small blood vessels and around the acini of seromucous glands. The pterygopalatine ganglion contains numerous VIP immunoreactive nerve cell bodies among non-reactive ones. Extirpation of the ganglion results in an almost complete disappearance of VIP-containing nerves in the nasal mucosa, while sectioning of the preganglionic nerve (Vidian nerve) does not affect the number of nasal VIP nerves. Hence the bulk of VIP nerve fibers in the nasal mucosa derive from cell bodies located in the pterygopalatine ganglion.

Many electrophysiological pharmacological and ultrastructural investigations point to the existence of non adrenergic non-cholinergic nerves in various tissues (Burnstock 1975). The atropine resistant vasodilatation in the nasal mucosa that results from electrical stimulation of Vidian nerve may indicate the occurrence of such nerves (Malm 1973a Ånggård 1974). One obvious candidate for this type of nerves is the system of vasoactive intestinal polypeptide (VIP) containing nerves. Several recent studies have shown VIP nerves to be widely distributed in the body (Bryant et al 1976 Larsson et al 1976 Alm et al 1977). There is accumulating evidence that VIP may act as neurotransmitter or modulator (Giachetti et al 1977 Emson et al 1978). We have previously reported on the presence of a rich number of VIP nerves in the upper respiratory tract of several mammals (Uddman et al 1978 1979). In these studies we found evidence for a local origin of tracheo-

bronchial VIP nerves since VIP-containing nerve cell bodies were regular constituents of intramural ganglia. In the nasal mucosa on the other hand no such cell bodies were detected. We now wish to present evidence that the VIP nerves innervating the nasal mucosa originate in the pterygopalatine ganglion.

## MATERIAL AND METHODS

Thirteen cats of either sex were anaesthetized by intracardial injections of sodium pentobarbitone after induction with diethylether. In 1 of the cats the pterygopalatine fossa on one side was exposed through a transpalatal approach (Malm 1973b) and the pterygopalatine ganglion a proximal segment of the posterior nasal nerve and a distal segment of the Vidian nerve extirpated and processed for immunohistochemistry (see below). Two of the denervated cats were killed two days after the operation and nine cats one to three weeks after the operation by exsanguination during pentobarbitone anaesthesia. In the remaining two cats unilateral extirpation of a short segment of the Vidian nerve was performed during shortlasting anaesthesia. These cats were killed three weeks after the operation. In all cats specimens were taken from various locations in the nasal mucosa (septum maxillare turbinal ethmoturbinal and nasoturbinal area).

Grant support was received from the Swedish Medical Research Council (04X 4000).



Abb. 1 (a) Zusammengepreschter Ansatzteil aus der Stria vascularis des Falles 1) Erste Windung: P, Prozess spiralis, E, flaches Rechteckteil; darunter unmittelbar angrenzend Ligamentum spirale (L). Im oberen Anteil besteht noch ein massives Striaödem (P) Insgesamt aufgehobene Striamorphologie und Fehlen sämtlicher Kapillaren. 460 (b) Zweite Windung. Über der gesamten Striaausbreitung schweres Ödem mit Verlust aller charakteristischer Striastruktur. 460

den Befunden bei der experimentellen Masugi-Nephritis den Einfluß von Nierenerkrankungen auf das Innenohr zu erhellen.

#### MATERIAL UND METHODIK

Die Felsenbeine zweier Patientinnen mit Alport-Syndrom wurden in einem Falle 1,5 Stunden post mortem, im anderen Falle 45 Mi-

nuten post mortem transmeatal nach Herausnahme des Trommelfelles des Sterngügels und nach Eröffnen des runden Fensters durch Perfusion mit 3,5%igem eiskühlem Glutaraldehyd perfundiert. Nach der Sektion

Für hervorragende technische Mitarbeit danke ich Frä. S. Linsenköhl.



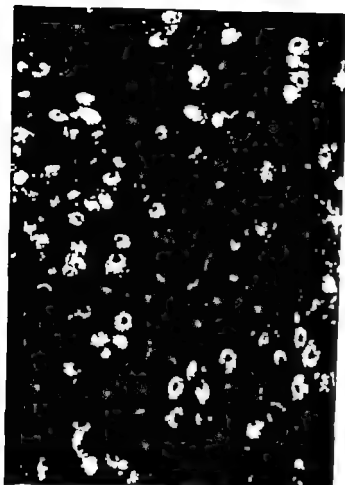


Fig 3 Pterygopalatine ganglion. Numerous VIP-containing nerve cell bodies throughout the ganglion. Note also the presence of immunoreactive nerve fibres among the cell bodies ( $\times 150$ )

epithelium around blood vessels and around the acini of seromucous glands (Fig 1)

The posterior nasal nerve contained a large number of coarse VIP immunoreactive nerve fibres (Fig 2). Occasionally VIP immunoreactive nerve cell bodies were noted within the most proximal segment of the nerve trunk.

The pterygopalatine ganglion contained numerous fairly large cell bodies displaying a varying degree of VIP immunoreactivity dispersed among non reactive ones (Fig 3). Immunoreactive nerve fibres were regularly found within the ganglion. In the most distal segment of the Vidian nerve adjacent to the ganglion a few VIP immunoreactive nerve cell bodies and nerve fibres were sometimes found. More proximal segments of the nerve were devoid of VIP immunoreactivity. Uni-

lateral extirpation of the pterygopalatine ganglion resulted in an almost complete disappearance of VIP nerves in the nasal mucosa of the same side as studied one to three months after the operation (Fig 4a). The number of VIP nerves in the contralateral side was visibly affected (Fig 4b). Two days after extirpation of the ganglion a rich number of fibres could still be observed in the nasal mucosa. Unilateral extirpation of the Vidian nerve did not affect the number of immunoreactive nerves in the nasal mucosa.

## DISCUSSION

The nasal mucosa has previously been shown to contain an extensive supply of acetylcholinesterase positive nerve fibres forming plexa in subepithelial connective tissue around small blood vessels and around glands (Dahström & Fuxe 1965; Ishiguro & Densert 1974; Grote et al 1975). The adrenergic nerves to the nasal mucosa seem to have their origin in the sympathetic ganglia (Ånggård and Densert 1975) while the cholinergic nerves reaching the mucosa seem to emanate from the pterygopalatine ganglion (Grote et al 1975).

The present results on the occurrence and distribution of VIP immunoreactive nerves in the feline nasal mucosa confirm previously reported findings (Uddman et al 1978). VIP fibres were distributed in the subepithelial connective tissue around blood vessels and around the nasal glands. VIP is a potent vasodilatory agent (Said & Mutt 1970) and is known to stimulate the secretory activity of various exocrine glands (Said & Mutt 1970; Konturek et al 1976). It is therefore conceivable that nasal VIP nerves are involved in the regulation of local blood flow and of mucous secretion.

A rich number of coarse VIP nerve fibres were found in the nerve distal to the pterygopalatine ganglion (posterior nasal nerve). The

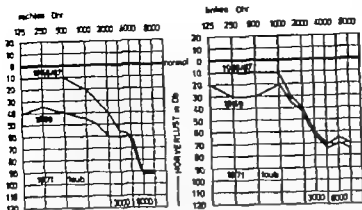


Abb. 4 Tonschwellenausbogenogramme 1966, 1969 und 1971. In den letzten Lebensmonaten war die Patientin taub

der Stria vascularis, aber nur eine geringfügige Degeneration der Prominentia spiralis. Lediglich im Bereich der dritten Windung fanden sich noch Bezirke von epithelähnlichem Aufbau mit weitgestellten Gefäßen. Der Flüssigkeitsraum des nur mehr spärlich vorhandenen Ligamentum spirale erstreckte sich bis unmittelbar unter die Epithelreste (Abb. 1a, b). Die Reissner'sche Membran war intakt. Im Bereich des Limbus spiralis imponierte ein Kondensat von PAS-positivem Material an der Oberfläche der Interdentalzellen. Auch die Tectorialmembran färbte sich intensiv PAS-positiv in ihren Randbezirken an. Das Cortische Organ wies in allen Windungsanteilen einen vollständigen Verlust der inneren und äußeren Haarzellen auf. Die Tunnebradialfasern fehlten in den Serienschnitten (Abb. 2). Das Ganglion spirale ließ eine Degeneration der Nervenzellen erkennen, wobei die umhüllenden Mantelzellen erhalten blieben und somit häufig keichförmige Areale zu erkennen waren (Abb. 3). Tonaudiometrisch bestand bei dieser 38-jährigen Patientin zum Todeszeitpunkt eine beidseitige Taubheit (Abb. 4).

#### Fall Nr. 2

Bei dieser 42-jährigen Patientin mit familiärem Alport Syndrom war mehrere Jahre vor dem Tod durch eine Nierenbiopsie, durch die klinische Symptomatik einschließlich eines langsam zunehmenden Hochtonverlustes und einer Linsentrübung die Diagnose bestätigt

worden. Die Patientin verstarb im Zustand der kompensierten Niereninsuffizienz an einem akuten Herzinfarkt. Sie erhielt während der gesamten Erkrankungsjahre keine innenohrtoxischen Pharmaka. Die Nierenbiopsie 1974 ließ im Bereich der Nierenglomerula noch eine Verbreiterung der Basalmembranen sowie eine Verschmelzung der epithelalen Zellausläufer erkennen, die nach David et al. (1966) und nach Zollinger & Mihatsch (1978) kennzeichnend für glomeruläre Veränderungen beim Alport Syndrom sind (Abb. 5a, b). Zum Zeitpunkt dieser Nierenbiopsie wies die Patientin eine äußerst geringgradige vorwiegend im Hochtonbereich gelagerte Innenohrschwerhörigkeit mit deutlichem Seitensunterschied auf. In den folgenden Jahren verschlechterte sich die Nierensituation und gleichzeitig kam es von einer geringgradigen Schwerhörigkeit zu einer mittelgradigen wiederum sehr unterschiedlichen Schwerhörigkeit, dabei war die stärkste Zunahme des Hörverlustes im Hochtonbereich vorhanden (Abb. 6).

Die Untersuchung der Nierenglomerula zum Todeszeitpunkt ließ eine weitgehende Degeneration und Obliteration erkennen. elektronenmikroskopisch waren die meisten Gefäße durch eine enorme Proliferation der Epithelien und Verdickung der Basalmembranen eingengt oder obliteriert (Abb. 7).

Die Stria vascularis war in allen Windungsanteilen voll erhalten, ausschließlich im Bereich der Reissner'schen Membran und unmittelbar

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Abb. 9(a) Corti-Organ der apicalen Windung. Degenerative Veränderungen der inneren Haarzelle (IHZ) bei normaler Struktur der äußeren Haarzellen (AHZ). 485 (b) Corti-Organ der Basalwindung. Äußere Haarzellen intakt, innere Haarzelle pyknotisch (X) Sinneshaare einer äußeren Haarzelle haften an Tectoralmembran.  $\times 485$



Abb. 10 Ausschnitt aus dem Ganglion spirale der Basalwindung. Normale Struktur fehlende Myelinisation ist nicht pathologisch. 493



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## DISCUSSION

- Klemen to Arnold. Emphasizes scepticism regarding the dilatation theory in Meniere and other conditions. Temporal bone histology requires an equally careful preparation whether for light or electron microscopy.

Grand Terminal Extension

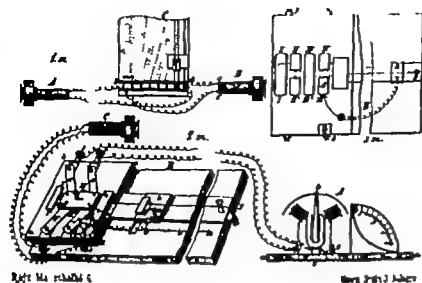


Fig. 3 Telephone-like equipment for objective measurement of hearing.

president and organizer of the Collegium meeting here in Budapest

In brief I should like to mention a few data from the history of Hungarian Otolaryngology

Endre Hógyes was professor of pathophysiology in Kolozsvár and in Budapest

(Fig. 1) His life extended from 1847 to 1906. In 1880 the associated movement of eyes and their connection with the actions of the semicircular canals was investigated and described by Hógyes. He performed the first experiments for objective measurement of hearing by means of a telephone-like electric apparatus (Figs. 2 and 3)

Jan Czermák was professor of physiology in Krakow in Budapest and later in Prague (Fig. 4). He was not a Hungarian and he did not speak in our language. But during his stay in Budapest the examination of larynx by a mirror was discovered by him and also the examination of the epipharynx by means of this mirror (Fig. 5). The first photograph of the larynx was also taken by Czermák.

Not far from Budapest in Albertirsa Ádám Politzer was born in 1835 (Fig. 6). Today we can still find a memorial tablet on his house where he was born. This tablet was placed by the Hungarian and Austrian Societies. Politzer's scientific activity in otology first of all in histopathology of the ear is well known. The Politzer balloon is used all over the world.

The next great name which must be mentioned is Robert Bárány. The Bárány family lived in Rohonc but his parents left Hungary for Vienna.

Bárány's life and scientific works are well known from their beginning to the present.



Fig. 4 J. Czermák

Table 1. Summary statistics for puretone AC thresholds

| Frequency (kHz) | Right ear |      |        |      | Left ear |      |        |      |
|-----------------|-----------|------|--------|------|----------|------|--------|------|
|                 | N         | Mean | Median | S.D. | N        | Mean | Median | S.D. |
| 0.5             | 1 203     | 25.2 | 22.7   | 13.4 | 1 203    | 25.8 | 23.5   | 13.4 |
| 1.0             | 1 206     | 36.3 | 36.3   | 15.2 | 1 204    | 36.9 | 37.7   | 15.8 |
| 2.0             | 1 204     | 52.1 | 53.6   | 14.3 | 1 203    | 54.1 | 55.4   | 13.9 |
| 3.0             | 1 203     | 64.9 | 63.4   | 13.8 | 1 198    | 64.2 | 64.4   | 13.4 |
| 4.0             | 1 195     | 68.3 | 68.1   | 14.4 | 1 195    | 69.2 | 68.6   | 13.6 |
| EN              | 1 184     | 67.4 | 68.2   | 20.2 | 1 182    | 68.5 | 69.4   | 19.1 |

is that the reflex plane is divided by two intersecting line segments: apparently the slope of the line segments and the point of intersection are determined by inspection not by any formal optimisation method. This makes statistical interpretation difficult.

Johansen et al (1976) stated that ARTs for puretones in patients with cochlear deficits are largely independent of the audiometric threshold, and they queried the logic behind the Niemeyer-Sesterhenn prediction formula. However they showed a clear positive association between average hearing loss and the ART for white noise but they did not express this in terms of a linear regression equation. They showed a scattergram for hearing loss against the difference between puretones and white noise ARTs which showed very little association. It may therefore be inferred that inclusion of the puretone average ART in a prediction equation may not contribute to the prediction accuracy and may even degrade it.

Keith (1977) reviewed the work of Niemeyer & Sesterhenn and Jerger et al noting that audiogram slope prediction by reflex methods is equivocal and that prediction methods based on white noise reflexes were as accurate as those which also included puretone ARTs.

Hall (1978) compared two versions of the Jerger predictor with an unpublished regression predictor reported by Baker & Lilly (1976). The predictive accuracy of the three methods was similar and decreased systematically as a function of increasing age. The performance of the Jerger predictors was de-

graded in the presence of minor abnormalities of the tympanogram.

In a comprehensive report Jerger et al (1978a) examined the effect of age degree of hearing loss and audiogram configuration upon the ARTs to puretone and wide-band stimuli. They found that all three variables influenced the reflex relations and that their effects were interactive. Jerger et al (1978b) reported the effects of age on reflex prediction. They found that predictive accuracy was substantially better in a group of children than for either young or old adult groups.

#### Present study

We attempted to model existing and new schemes in order to evaluate the efficacy of ARTs in hearing threshold determination of a specific population—adults with presumed noise-induced hearing loss (NIHL). From this group 1207 patients referred to our clinic (MSH) for assessment between January 1975 and January 1978 with reliable puretone audiograms and bilateral reflexes to wideband noise were selected as subjects. Data were analysed on the University of Toronto IBM 3033 computer primarily using the Statistical Package for the Social Sciences (SPSS).

**Reflex measurement methods.** The ART measurements considered here were obtained using standard clinical methods. Contralateral ARTs were obtained to tonal stimuli at frequencies of 250 500 1000 2000 3000 and 4000 Hz, and to unfiltered low pass and high pass filtered white noise. The 3 dB point was 600 Hz for both filters. The units for the tonal



Gross Torna, ad Extremum

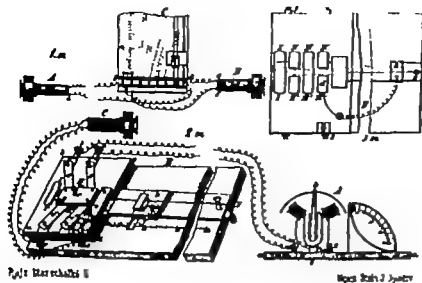


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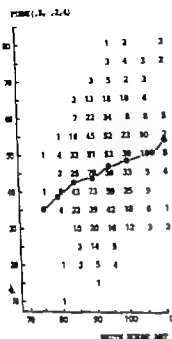


Fig. 3. Cross-tabulation of average ART at 0.5, 1, 2 and 4 kHz and the white noise ART. Circles are the conditional mean average PTT.

and thereafter shows a rapid rise. The white noise ART increases fairly linearly throughout the loss range. The convergence principle is therefore valid for losses up to 70 dB. These results are comparable with those obtained by Jerger et al. (1978a).

(e) *Prediction of audiogram features from ART measurements.* Most published reports on prediction of hearing level from ART measurements consider average hearing losses. Such an approach arises from an historical perspective or because of an underlying presumption that average hearing losses will somehow relate better to ARTs than will single puretone hearing loss measurements. The results in Table V show that this is not entirely true, but the noise ART/average loss correlations are among the highest in the table.

The predictability of the average puretone loss at 0.5, 1 and 4 kHz by single ARTs was examined. From Table V it can be seen that the white noise and high pass noise ARTs correlate equally well. The former was

Table VI. Conditional statistics analysis of variance and regression data for the average PTT threshold at 0.5, 1, 2 and 4 kHz by ARTWN. PTAVE statistics.

| ART value | Mean | S.D. | N   |
|-----------|------|------|-----|
| 75        | 34.4 | 1.5  | 6   |
| 80        | 39.0 | 10.7 | 23  |
| 85        | 42.8 | 9.1  | 165 |
| 90        | 43.8 | 10.3 | 357 |
| 95        | 46.3 | 10.6 | 350 |
| 100       | 47.9 | 11.0 | 186 |
| 105       | 49.5 | 10.5 | 80  |
| 110       | 53.9 | 14.2 | 25  |

Analysis of variance:

Between groups:  $p$  less than 0.0001

Linearity:  $p$  less than 0.0001

Nonlinearity: NS

Simple linear regression:

$PTAVE = 7.6 + 0.4 \cdot ARTWN$

Standard error of slope: 0.048.

Residual mean square: 109.7

F ratio 72.9  $p$  less than 0.0001

Number of cases: 1192.

selected for detailed study because of its higher incidence (Table II).

The cross-tabulation of the average behavioural threshold at 0.5, 1, 2 and 4 kHz versus the white noise ART is replotted in Fig. 3 with the white noise ART as the abscissa. It can be seen from Fig. 3 that the conditional mean average loss increases linearly with increased ART (conditional statistics and regression data are given in Table VI). The key observation is that the range of the conditional means and therefore the approximate range of prediction, is only about 20 dB and the standard error of prediction is greater than 10 dB at any value of white noise ART. Therefore although the white noise ART does permit prediction of average hearing losses, the clinical utility of this prediction is extremely limited.

Clearly the conditional average loss distributions will depend upon the population under study. If this were restricted in range then the range of prediction would also be restricted. We do not consider this to be a serious objection to these data. The range of average losses

years ago (Fig. 7). His findings in the physiology of hearing are well known since they became public property.

I should now like to say a few words about the present situation of ORL in Hungary. At present we have four universities for medical students and one postgraduate medical school also with an ENT department for doctors and specialists.

Today there are in Hungary more than 450 ENT specialists among the 23 500 physicians.

To round off my opening speech I have the honour of mentioning that the oldest member of our Collegium is Gy. Keleman, as I know he is now 90 years old. Let me convey to him from my heart—in the name of the Collegium membership and also of all Hungarian otolaryngologists—our best respect and gratitude.

*L. Székán*

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## OBSERVED THRESHOLD

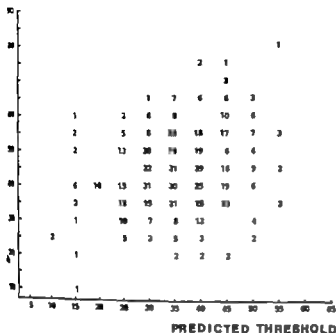


Fig. 5 Cross-tabulation of observed versus PTT (ordinate) predicted average PTT (abscissa) from the Niemeyer-Sesterhern predictor

### Comparative assessment of predictive formulae

The predictive performance of the Niemeyer-Sesterhern formula was compared with prediction using white noise alone and with a multiple regression equation derived in this study. The Niemeyer-Sesterhern formula incorporates the 4 kHz ART. Accordingly the formulae were compared in a sub-population of 719 patients having all appropriate reflexes.

In Fig. 4 the difference between the average ART at 0.5, 1, 2 and 4 kHz and the ART for white noise is cross-tabulated against the average ART minus the average puretone threshold at those frequencies. This corresponds to Fig. 3 in Niemeyer & Sesterhern's original report (1974).

The solid line in Fig. 4 corresponds to their prediction equation which states that the abscissa is 2.5 times the ordinate. The data of this study do not follow that relationship: the bivariate distribution is clearly more horizontally oriented than that required by the formula. A substantial number of points are out

side the 20 dB limits, especially for small values of the abscissa.

One way of visually assessing the performance of a prediction equation is to cross-tabulate the observed and predicted values. In Fig. 5 this cross-tabulation is shown for the Niemeyer-Sesterhern predictor. If the predictions were perfectly accurate all the points would lie on a diagonal line with unit slope. The enormous scatter is clear from the figure. There is a tendency for predictive errors to be positive at low predicted values and negative at high predicted values. As well as a great deal of variation there appears to be systematic bias in prediction. These results differ in both location and scatter from those of Miller et al. (1976) but that study is both small-sample and contains a normal-hearing group.

Carrying out a simple linear regression of the four-frequency average hearing loss on the white noise ART in this sub-population gave a predictive formula shown in Table VII: the correlation coefficient was 0.22. The cross-tabulation of observed and predicted values is

lins with the complement system etc. but the acute or chronic inflammation is not enough to investigate alone by the methods of classical immunology

(4) In our field some misinterpretation is also observable. The immunological reactions or other factors of the defence mechanism are neglected or on the contrary overestimated e.g. in cases of acute middle-ear inflammation or in indication of tonsillectomy

(5) Very serious problems arise from the fact that our minds are far more influenced—as was mentioned above—by the good or appar-

ently good effect of antibiotics than by real knowledge of the defence reactions of the body and very often we work against them. For instance the defence mechanisms are very frequently inhibited by the antibiotics. This is observable first of all if antibiotics are administered too early in the inflammation

I believe that the above-mentioned problems are very important. Therefore it would seem to be very useful to discuss these common problems of immunology and otolaryngology

necessary to achieve this involve completely different sets of variables.

The decreasing effectiveness of the white noise reflex as a predictor of average hearing loss in the high slope group and the production of completely different multiple regression predictor equations for the low slope and high slope groups, make the use of ART prediction procedures even more problematic.

One possible solution to this is to develop prediction procedures in two stages: the first consisting of the prediction of slope and the second a prediction of severity. To this end the ability of reflex measures to predict audiogram slope was examined. The measure selected was the ART in high pass noise minus the ART to low pass noise. This was both correlated and cross-tabulated against the observed audiogram slope as measured by the difference between the 4 kHz and the 1 kHz puretone thresholds. The correlation coefficient obtained was only 0.22 and the cross-tabulation showed no appreciable diagonalization. It is concluded that this ART measure cannot serve as a useful basis in a multistage prediction process.

One final and simple examination undertaken attempted to relate values of PTT to ARTs for PTs. It has often been pointed out that if a recorded PTT were worse than a measured ART for that frequency the PTT must be in error (Alberti 1970). However the literature is not clear how much higher the ART must be than the PTT before the latter can be considered reliable. We investigated what level of PTT had a less than 10% chance of actually being exceeded for different decade levels of ART. The results for 2 kHz are shown in Fig. 8 where it can be seen that if the ART is 100 dB or greater the PTT has a 90% probability of being at least 35 dB better whereas if the ART is 90 or 80 then the PTT may come to within 25 dB of the ART. If it comes closer the patient should be re-examined audiometrically before accepting the result.

The results of this study are disappoint-

ing. In adults objective predictive techniques are potentially of greatest use in medico-legal settings, particularly with large populations such as in screening industrial hearing loss claims. It is exactly such a population which has been tested and where all simple techniques appear to have no real quantitative value. ART measurement has a qualitative role to play both in site of lesion testing and in identification of hearing loss but no accurate quantitative predictions about hearing loss can be based upon it.

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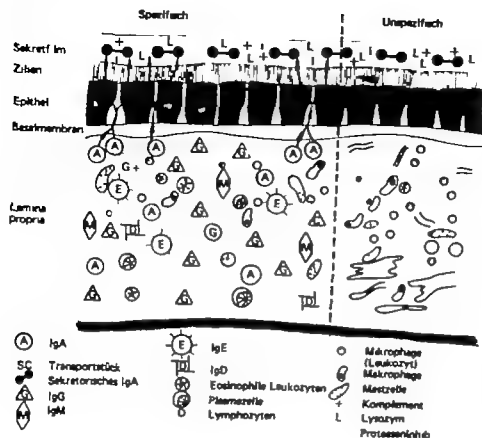


Fig 1 Synopsis of some specific and non-specific factors influencing the defense mechanism of the respiratory mucosa

*Lysozyme* is the longest known protective enzyme in nasal secretion. It attacks glucoside compounds in polysaccharides and mucopeptides such as occur in the cell wall of Gram positive bacteria. The action of lysozyme causes the step-by-step disintegration of the bacterial cell walls—probably due to the simultaneous action of complement (Glynn 1966).

Gram-negative bacteria incidentally are protected from lysozyme attack by a lipopolysaccharide component in their capsules (Mims). According to recent studies lysozyme which is bacteriostatically bactericidal and bacteriolytically active is produced among other things in the mucosa glands. It can also be detected in large amounts in the police cells (macrophages and microphages). There are indications that lysozyme has a synergistic effect not only with complement but also with immunoglobulin A.

Yet another aspect is of interest in this context: the lysis of the bacterial cell wall caused by lysozyme is not carried down to the mono-

saccharide stage but only to the larger decomposition complexes (penta-*N*-acetylglucosamine). Further decomposition of the cell wall fragments including complete hydrolysis is then carried out by various glucosidases which are also present in the nasal secretion. This is important because cell wall substance which is only partially decomposed is highly immunogenic.

Lactoferrin also produced in the respiratory mucosa and also detected in neutrophilic leukocytes has a protective function against microorganisms as well. In vitro it apparently inhibits the growth of bacteria such as *Staphylococcus albus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by removing iron ions from the nutrient medium.

The lysozyme group acts against bacteria, but not viruses. Interferon by contrast also present in nasal secretion is characterized by considerable antiviral activity. This protective protein is formed by very many cell types—including epithelial cells—within a few hours

2) The analysis of 25 000 tone threshold audiograms made after hearing losses and noise exposure confirms the data gained in practical work.

3) Experimental studies show—as a consequence of the 85 dB(A) noise level—a lack of hearing recovery which is the ultimate cause of the permanent hearing defect.

## ZUSAMMENFASSUNG

Wie die Jahrzehntealte ärztliche Begutachtung zeigt, bestehen stärkere Hörschädigungsrunden nicht erst ab 90, sondern schon ab einem Lärmpegel von 85 dB(A). Diese Erfahrung wird durch eine Analyse von 25 000 Tonschwellenaudiogrammen und durch das Ergebnis experimenteller Gehörstörungstest bestätigt.

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Austria

## DISCUSSION

*Spencer to Schm. etc.* Your first slide showed that there is a very little barely significant increase of damage risk between noise levels of 85 and 90 dB(A), whereas much greater increases exist between 75 and 80 dB(A) or 90 and 95 dB(A). What therefore is the reason I propose 85 dB(A) as permitted noise level limit rather than 90 dB(A) as it is in Germany and Switzerland? The barely significant increase in damage risk will probably not

justify the enormous costs of reducing industrial noise emission by 5 dB.

*Tonndorff to Schm. etc.* W. In the US would also be happier with 85 dB(A) maximal level. However as you are fully aware this is not a scientific but a political decision. At the international level at the ISO (we have meeting in Delft) Sept. 26) I am happy to report (1) that the new standard will no longer give fixed values like 90 dB(A), but only guidelines so as to force the administrative authorities *agreed* into action, (2) the three speed frequencies will be increased to four 5 1.0 2.0, 3.0 kHz which will make the index more sensitive.

*Schm. etc. (Reply).*

The contributions made to the discussion, for which I am most grateful, can be answered together.

Of course the problem of fixing the critical intensity value at a certain point is also a political question in that it touches upon the financial aspect. But as it involves the question of maintaining the health of the working population, it must be seen first and foremost as a medical one. I too encountered difficulties with the competent authorities when many years ago I wanted to introduce 85 dB(A) as the critical intensity value to be generally observed in Austria. What I demanded at that time was based only on individual observations! The results which are now submitted should make it easier for the medical profession to convince the politicians through factual material.

As concerns the effect of noise-induced damage on sound-hearing, let me say the following: For the maintenance of speech hearing neither the auditory threshold for 3 kHz nor that for 2 kHz is of any decisive significance. As I was able to show only recently on the basis of a larger number of patients examined, the speech discrimination capacity depends above all on the auditory threshold loss at 1.5 kHz in the case of typical noise-induced damage.



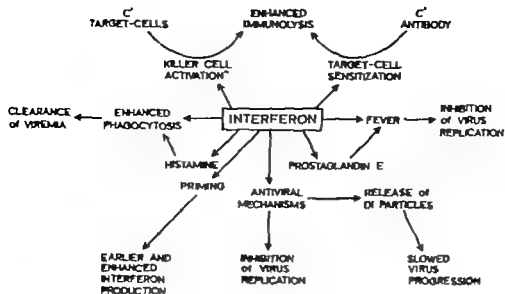


Fig 4 Involvement of interferon in host defense mechanisms (from W. E. Stewart II)

This is one of the most important unspecific biological effector systems. It also acts as a mediator in immune reactions and intensifies them (Fig 5). Complement is an enzyme system consisting of nine individual factors whose individual components are present as inactive precursors in blood, tissue and secretions. The complement system can be activated by immuno-unspecific and by immuno-specific mechanisms. It then proceeds similar to a chain reaction which passes either via the classical path (C1-C9) or by a shortcut, the alternate path, beginning at C3, resulting in inflammation and cell destruction. The so-called C3 shunt is triggered by the properdin system (serum protein plus complement plus  $Mg^{++}$ ) which is directed against Gram-nega-

tive bacteria and a few types of virus (Schwartz & Heide 1970). Without being able to go into further detail, it should be mentioned that complement fulfils four anti-microbial functions in the defense of infections: (a) activation of the inflammation reaction, (b) attraction of leukocytes and plasma factors to the site of conflict with the invader (including the release of anaphylatoxin and the resultant degranulation of the mast cells), (c) activation of phagocytosis, and (d) destruction of micro-organisms and/or contaminated cells.

The aim of the complement system is to make holes in or destroy the membranes of the foreign cells. This is due to an interaction of the component constituents C5-C9 with specific phospholipids in the cell membrane.

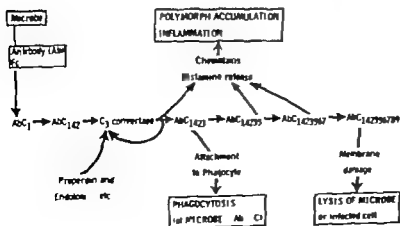


Fig 5 Complement activation sequence and antimicrobial actions (from C. A. Mills)

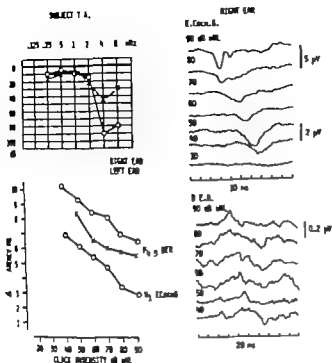


Fig 3 Typical example of the effects of sensorineural high-frequency hearing loss on BER responses. Compare the results with those in Fig 2.

measures to latencies. In Fig. 1 are presented the results from one normal subject. In all normal cases the latency/intensity curves of ECoG peak (N) and BER wave  $P_{1-2}$  were parallel and showed a latency difference of

about 4 ms (see also Fig. 5). At high intensity levels the latency of the first peak (P) in BER could be measured and was found to correspond to that of ECoG N, but for middle and low intensities it was impossible to detect.

#### Cochlear/retrocochlear cases

On the basis of ECoG results alone we found that the strongest indicator of a retrocochlear disorder was a threshold level better than could be expected from the subjective hearing revealed by the audiogram. Statistical studies relating the click threshold at ECoG to the subjective audiogram showed that it is a good indicator of subjective audiometric thresholds for middle frequencies (Eggermont, 1976). In all the subjects in this study we compared the ECoG click threshold with the best audiometric threshold for frequencies of 1, 2 and 4 kHz. The ECoG click threshold was better in 4 out of the 14 patients with a confirmed tumour; such a phenomenon was never observed in the group with cochlear impairment or in the normals. The pattern of ECoG response in itself did not appear sig-



Fig 4 Results from Meniere patient presenting with recruitment. Although the threshold is elevated the first peak (P) in BER recordings appears clearly.

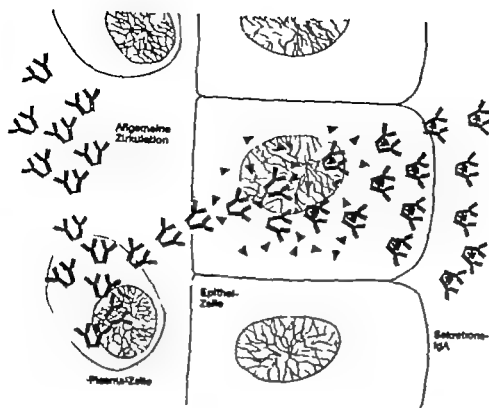


Fig 8 Scheme showing production of IgA and its synthesis to secretory IgA (from O G Bier).

the invader is immunospecific or -unspecific. Mention of this group of agents brings me to a special field with which Prof. Hochstrasser and his working group at our Munich clinic are involved. Nevertheless the explanation will be very brief.

The protease inhibitors are themselves proteins which due to their structure can interact with various proteolytic enzymes to form complexes. They constitute a type of regulator for enzymatic activity. In the case of humoral

and cell linked immune reactions the result is cell destruction together with an associated release and activation of protein-splitting enzymes the proteases. If the proteases could act in an unchecked manner the infected organism would be partially digested at the site of the conflict and thus damaged. The protease inhibitors prevent this from happening.

According to the current stage of research three groups of protease inhibitors can be differentiated.

(1) High molecular acid instable inhibitors (Serum-inhibitors) present substantially in the circulatory system and the interstitium. They probably pass over into the secretion only by transudation.

(2) Another inhibitor group can pass through tissue and is secreted easily. It is found in many mucous membranes. These low-molecular inhibitors prevent mucosa damage by proteases from the body's own cells as well as to proteases released directly and indirectly by microorganisms. They are acid-proof and make up approximately 80% of the anti-tryptic activity of the secretion. The precursors

## BARRIER

### EPITHELIUM AND BASAL MEMBRANE

- |     |                                  |
|-----|----------------------------------|
| I   | CILIA                            |
| II  | EPITHELIAL FORMATION             |
| III | BASAL MEMBRANE                   |
| IV  | CRITICAL THRESHOLD OF RESORPTION |

Fig 11

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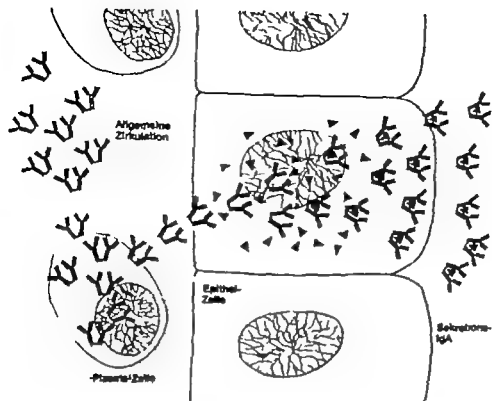


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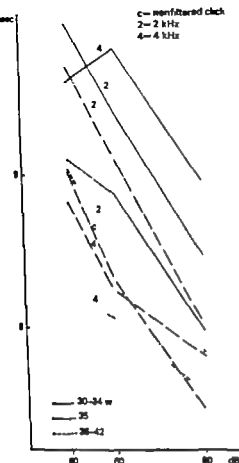
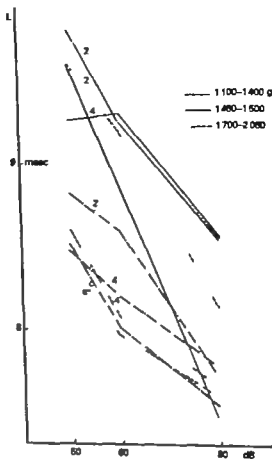
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## BARRIER

### EPITHELIUM AND BASAL MEMBRANE

- I CILIA
- II EPITHELIAL FORMATION
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- IV CRITICAL THRESHOLD OF RESORPTION

Fig 1 Latencies  $J$  as function of gestational ageFig 2 Latencies  $J$  as function of weight.

Respective influence of age and weight  $J$  wave latency for non-filtered clicks was compared in the four conditions where the variables were as follows: gestational age alone; weight alone; weight and age in two groups; weight and age in three groups.

Gestational age proved to be the most important factor, but the influence of weight was greater for the lower weights and with lower intensity stimulation, that is 60 and better still 80 dB.

#### *Little opportunity to observe pathological recordings*

Our experience was limited to one case of a full-term baby weighing 3300 g admitted to hospital with pyocyanic septicaemia treated with amikacin. This baby died. The B.S.R.A.

recording performed a week before death was completely flat.

#### *A single extended study in ideal conditions of one baby examined three times*

1200 g, 35th week  
1500 g, 38th week  
2050 g, 42nd week

The quality of the recordings made it possible to measure the latencies of  $J$ ,  $J_{II}$ ,  $J$  and  $J_{VI}$  waves. Latency at 2050 g reached at the 42nd week, full-term, was not the same as for an adult but AP- $J_V$  was close to it.

### CRITIQUE

The major criticism of the present study is the limited number of observations made. In fact

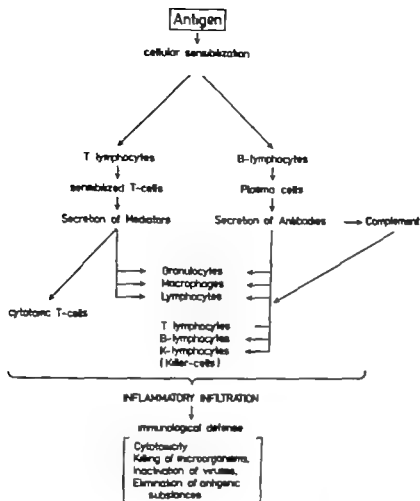


Fig. 11 Functional relations between B cells and T cells (from K. Resch)

This immune body can also be formed in the mucosa but acts as the very largest of all immunoglobulins especially intravascularly.

Only with elevated vascular permeability does IgM penetrate into the tissue and thus supplement the IgM concentration which may already be present there. It is found only in trace amounts in nasal secretion. Only in individuals who cannot produce any IgA can it take over its place and function in the secretion and mucosa area.

In the first line of defense IgA is undoubtedly the most interesting and most important immune body. Owing to its combination with the secretion it is also termed a mucosa-antibody which together with the secretion layer forms an antiseptic protective film.

IgA is the immunoglobulin of the mucous membranes and has the task of preventing accumulation, adhesion and invasion of micro-

organisms and other antigens on and in the mucosa. Unlike other immunoglobulins it requires a relatively complicated composition to be able to perform its protective function on the mucosal surface. A monomeric form of IgA is formed in the plasma cells especially in the mucosa. The monomers join together to form dimers and polymers in the lamina propria of the mucosa and are then transported in the direction of the mucosa surface in particular adjacent to the mucosal glands (Fig. 8).

A so-called secretory piece which is produced in the epithelial cells (Tomasi 1976) must appear however to activate the surface protective function. Only the combination of the polymeric IgA with the secretory piece form the secretory IgA which is active on the mucosa surface or in the secretion. The secretory piece apparently facilitates the passage of the IgA polymer through the epithelium and

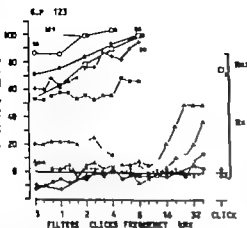
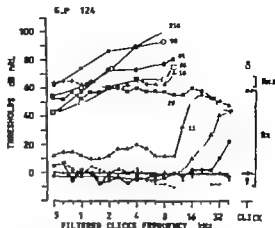


Fig. 1. Frequency threshold curves of the responses recorded at the round window for guinea pigs at various times indicated in days (small characters) after the beginning of the first aminoglycoside treatment (day 0). Note the



rapid evolution during the first treatment (Rx1: 450 mg/kg/day) and the small changes during and after the second treatment (Rx2: 20 doses), particularly for GP 124 (right).

while the other ear not involved by the procedure, served as a control at time of sacrifice of the animal. The surgical procedure as well as the method of stimulating and recording from this electrode in awake animals have been described in detail elsewhere (Aran & Erre, 1979) and used in various studies (Aran & Darrouzet, 1975; Aran & Cazals, 1978). As usual the auditory nerve compound action potential (CAP) thresholds to filtered clicks from 0.5 to 40 kHz are determined (CAP audiograms) the intensity levels being referred to the mean threshold of normal responses (dB aRL). Although we normally use a Bruel & Kjaer condenser microphone cartridge (4134) directly coupled to the ear as a transducer in these experiments as soon as the thresholds became elevated we had to use a TDH 39 Telephonics earphone in order to obtain higher sound levels although the frequency of the transient stimuli could not then exceed 10 kHz. Responses to the unfiltered click using this earphone were also recorded from the very beginning of the experiments so that patterns of the responses and input-output amplitude and latency functions could be monitored over the entire experiment. As usual for the recordings we automatically used averaging

and clicks of alternate polarity to cancel out any microphonic potential.

#### Effects of the first treatment

These 5 implanted guinea pigs having normal click-evoked responses and CAP audiograms received a first treatment of amikacin identical with that used in our earlier studies (Aran et al. 1979; Cazals et al. 1979b) i.e. 14 intramuscular injections of amikacin (daily except during week-ends) at doses of 450 mg/kg. Responses were monitored every 2 to 4 days before and also during the treatment, then with longer time intervals between tests after them.

For all the guinea pigs CAP audiograms started to alter already at the end of the first week of treatment, beginning with an elevation of the high frequency thresholds (Fig. 1). By the end of the treatments all the CAP audiograms showed the same pattern with thresholds around 50–70 dB at 0.5 kHz and progressively increasing with the frequency up to 90–100 dB at 8 kHz.

Then shortly before or after the end of the treatment 3 guinea pigs died (at 14 and 20 days).

By this time the click-evoked responses were profoundly altered in all the animals



Cell-mediated immunity

some mediators in man

| Mediator                       | acting on                             |
|--------------------------------|---------------------------------------|
| Chemotactic factor             | Lymphocytes, Macrophages, Microphages |
| Blastic factor                 | Lymphocytes                           |
| Resistance-producing factor    | Macrophages                           |
| Interferon                     | Viruses                               |
| Inhibitory factor of migration | Macrophages                           |
| — of clon induction            | Lymphocytes                           |
| — of proliferation             | cells altered by antigen              |

(from E. Maccher)

Fig. 13 Some mediators in cell-mediated immunity (after E. Maccher)

the invader and according to the actual defense posture of the mucosa a partial or total mobilization of all mucosa defense possibilities is begun (Fig. 10)

The intercellular substance consisting of mucopolysaccharides and proteins constitutes the foundation of the lamina propria. This basic substance is a very variable colloidal system forming not only a mechanical barrier but also constituting a fundamental biochemical defense factor since all reactions and functions of the tissue must proceed via the basic substance—whether they be nutritive, hormonal, toxic, metabolic or inflammatory in inflammatory processes.

Unspecific and specific defense mechanisms are integrated in the lamina propria—as in the secretory film—although the active factors have a different composition compared with the secretion.

The unspecific local reactions include among other things the mobilization of the neutrophilic leukocytes, the activation of the macrophages and the initiation of the associated phagocytosis as well as the induction of inflammation in co-operation with the mast cells. The unspecific reactions however also include the activation of the complement system and the production of interferon.

The specific local defense system in the mucosa is guaranteed by humoral and cellular im-

mune reactions. In our special case of microbial penetration by way of the mucosa surface, a local immune reaction normally develops in which both humoral and cell-mediated immunological mechanisms mutually participate.

The many, sometimes very complicated details with which modern immunology confronts us cannot be dealt with within the scope of this paper. One should however mention a few immunological facts in this context in order to round off the topic.

The lymphatic cells play a decisive role in the defense against infection. The individual lymphocytes differ by a number of immunological properties. It is a well-known fact that a functional differentiation is made between two fundamentally different classes of lymphocytes: the B cells and the T cells. In some what simplified form the functional relation can be explained as follows: antigens in our case microorganisms which are recognized by the organism as being foreign result in sensitization (Fig. 11). In so doing both B and T cells are activated.

The activation of the B cells leads to the maturation of plasma cells (Fig. 12) which in turn produce specific antibodies. All cells of a clone and their offspring retain the capacity to synthesize at all times the same specific immunoglobulin directed against the antigen causing the sensitization. The formation of a new antibody takes about one week. If on the other hand immunoglobulins against an antigen are already available in the organism, however, this latency interval is shortened to an immune response of 1–2 days. In this immune response the B cells co-operate with the complement system and—similar to the T cells as well—with granulocytes, macrophages and other lymphocytes. It may be of interest that there are roughly  $10^4$  receptors for antigens on a single B cell (Mims!).

T cells (Fig. 12) constantly migrate through the tissue and thus through the mucosa as well. While on patrol they may encounter an invading antigen—in this case microorganisms. This contact

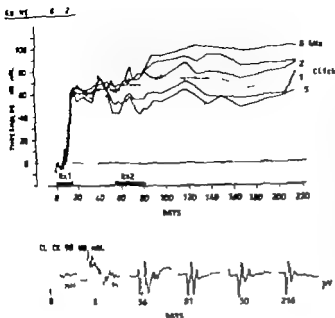


Fig. 3 Upper curves: Evolution of the thresholds to the various filtered clicks (from 0.5 to 8 kHz) and to the click ( ) during the entire experiment including the anidoxan treatments (Rx1 and Rx2). Note the relatively good low frequency thresholds. Lower trace: Click-evoked responses from the same guinea pig at various times. Traces as in Fig. 2. Peak-to-peak amplitudes indicated in microvolts in small characters. Note the disappearance of the early positive peaks during the second treatment (days 36 to 81). It is not obvious whether the final response (at days 150 or 216) is present or not as the responses at 0, 18 or 36 days.

the ears and from the auditory cortices (with contralateral acoustic stimulation) in GPs 124 and 117 (Figs. 4 and 5) while no response was recordable either from ears or from the auditory cortices of GP 123.

In GP 189 responses were remarkably constant both in pattern and amplitude from day 18 (implantation) to day 347 (sacrifice) (Fig. 6). Similar responses could be recorded in GP 196

(Fig. 7) while no response could be recorded from either the left and right ear of GP 195. However for this GP it was obvious either during electrode implantation for acute recordings or later when preparing the ears for histology that it had been suffering from bilateral otitis media (thickening of bone and presence of membranous material in the middle ears).

#### GUINEA PIG 117

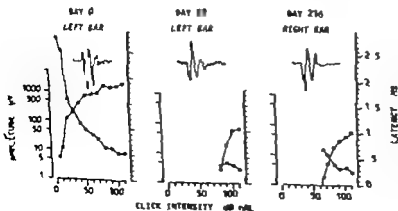


Fig. 4 Patterns of the click evoked responses at 90 dB nRL (upper trace) and input-output amplitude and latency functions at days 0 and 32 from the left ear and at day 216 from the right ear of GP 117 which received the same treatment (Fig. 3) as GPs 123 and 124. Traces as in Fig. 2.

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## DISCUSSION

**Sjogren to Aran.** In some of your animals you recorded acoustic potentials in spite of an apparently complete loss of hair cells and spiral ganglion cells. This is in fact very surprising and can only be explained by an acoustic activation of some unaffected vestibular elements. I would also question any correlation between the ECOG response and functioning cochlear neurons. The quantitative morphological evaluation of the cochlea is of course crucial for the interpretation of these results. Such quantitative critical evaluation is probably very difficult on the basis of serial sections and I think combined surface and EM technique would be better. In our experience it can easily happen that one finds distorted rudimentary hair cells in EM preparations of cochleas where in LM all hair cells seem to have disappeared.

**Berman to Aran.** It is very difficult to find an explanation for the described observation. If we assume that all hair

cells are destroyed and not merely damaged and still functioning, we have to think of vestibular transformation. This vestibular source of the potentials seems to be very improbable—otherwise we would find some potentials more frequently in completely deaf patients. One hypothetical explanation seems to be a sinister phenomenon we all know named electrophony. As it is based on electromechanical transformation it could also be possible that mechanoelectrical transformation produces an electrical stimulation of the nerve fibres.

**Gilson to Aran.** I ask whether any adaptation studies have been performed. This would distinguish between a receptor potential or an artificial microphone and a truly neural component.

**Beagley to Aran.** This response should perhaps be called "hair-cell loss acoustic response" rather than a cochlear response. Presumably the other cells of the organ of Corti are present?

**BM Johnstone** has postulated direct mechanical-stimulatory effect on the nerve fibres supplying the IHCs. It is possible that this is the origin of the response in the auditory nerve that Aran has described.

As part of the response has a short latency 0.3 ms, it suggests that some form of receptor potential is involved. It would be interesting to do d.c. recordings of the scala media to see if there is an endolymphatic d.c. potential present in the ears damaged with Amikacin.

**Bauer to Aran.** It would be interesting to know the frequency spectrum of the click stimulus. Assuming that it is response of the saccule, strong click may cause sudden dislocation of the stapes which might be a proper stimulus for the saccule. If this is so have you observed any movements of the eyes by nystagmography?

**Aran (Portmann repl.)**

Thank you for your comment. I, the absence of Dr Aran, I will try to do my best and invite you to our laboratory to see exactly what Dr Aran and his group do.

They carefully observed with light and electron-microscopy and they believe that the hair cells are destroyed.

The behaviour of the response proves that it is not an artefact. I thank the speakers for the ideas they gave to try to explain the phenomenon.

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sue proved to be a less good interpositum. The venous wall partly calcifies, is transformed and becomes stiff. A part of the fatty tissue necrotizes, another part forms, in some of the cases, undesirable granular tissue.

Before transplanting the materials we had attempted application of a low-energy helium-neon laser in order to observe how the irradiation of the low-energy laser affects the adhesion of transplantation. Unexpectedly the low-energy laser promotes the vascularization and adhesion of transplantation considerably. As a result of irradiation the transplant incorporates faster and vascularization also follows earlier.

We have also applied irradiation to close the perforation. The low-energy laser induced rapid epithelialization of the perforation.

We have applied irradiation to close experimental tympanic membrane perforations as well. The low-energy laser induced rapid epithelialization of the perforation. It was shown by the histological investigation carried out after the low-energy laser irradiation that no change arose. On the other hand oedema was formed at elevated energy levels.

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## DISCUSSION

*Portman to Ribáři.* I think that we have to avoid gelfoam for closure of the oval window. I 1964 I did 300 cases with gelfoam and the sensorineural hearing loss went up from 0.7% to more than 6%.

I think that we must recommend absolute avoidance of gelfoam—if it is not toxic by itself, which is not proved, it does result in this membrane which does not protect the labyrinth sufficiently against inflammation from pharynx and tubal region.

*Teleman to Ribáři.* Tissues acquire independence in late life as in embryonic periods.

*Ribáři (Reply).*

Thank you very much for your questions.

The gelfoam made very thin membrane but it has no toxic effect.

It depends on the method of sterilization.

An ideal material is Gelfoam and pericardium. Fat and vein are no good.

Table I Cytologic types of effusion

| Cytologic type        | Serous or seromucinous effusion |     | Mucoid effusion |     |
|-----------------------|---------------------------------|-----|-----------------|-----|
|                       | N                               | %   | N               | %   |
| Granulocytic          | 37                              | 65  | 69              | 58  |
| Monocytic-lymphocytic | 10                              | 18  | 20              | 17  |
| Mixed                 | 3                               | 5   | 11              | 9   |
| Few cells             | 7                               | 12  | 19              | 16  |
| Total                 | 57                              | 100 | 119             | 100 |

Cytologic type could not be determined.

sponse and the damage caused by immune complexes possibly present in the middle ear liquid

### Cellular Response

In secretory otitis media the cellular response may vary considerably. There are specimens both of the serous and of the mucoid type which contain only small numbers of inflammatory white cells whereas the majority of cases show considerable inflammatory response. In our material the frequency of specimens with a poor inflammatory response was 12% for the serous and sero-mucinous and 16% for the mucoid type respectively.

In the microscopic examination of smears stained with Wright's or Papanicolaou's stain and cytocentrifuged cell preparations stained with May-Grünwald-Giemsa (MGG) the effusion liquids were divided into three groups: a granulocytic type, a mononuclear cell type and a mixed type (Palva et al. 1978, 1979). In the first group we included all specimens in which 60% or more of the cells were polymorphonuclear leukocytes and in the second those in which the mononuclear cells exceeded 50%. The liquids in which polymorphonuclear leukocytes constituted between 50 and 60% of the cells formed the third mixed group. Table I shows that most of the effusion liquids were granulocytic, lending support to the view that ongoing infection is an important

etiological factor. Nearly 20% of the liquids were monocytic-lymphocytic.

In a further subdivision of the monocytic-lymphocytic specimens we used the recently discovered  $\alpha$ -naphthyl-acetate-esterase enzyme (ANAE) staining method to mark the T lymphocytes. This histochemical staining method has been employed in studies on human specimens by Ranki et al. (1976) and by Ranki (1978) after Möller et al. (1975) had discovered that in histological sections of mouse lymph nodes the T-cell areas were positive for this enzyme. In human blood 90% of lymphocytes which bind sheep erythrocytes express this marker while the surface Ig-expressive lymphocytes (B-cells) remain negative. A fraction of the null cells also expresses the marker but as their overall number is small their effect upon the total frequency of marker-positive lymphocytes is insignificant.

Table II shows the lymphocyte composition as evaluated in the cytocentrifuged specimens after incubation for 6 h to demonstrate T-cells with the ANAE. The effusion type, whether serous or seromucinous or mucoid, had been assessed on the basis of gross appearance and it can be seen that the percentage of T-cells in both liquids was comparable to that found in the blood, i.e. 60%. It should be borne in mind, however, that irrespective of the duration of the disease 10–15% of specimens from chronic effusions had very sparse cellular populations. Furthermore

Table II ANAE positive lymphocytes in effusion

| Effusion type           | Frequency of ANAE positive lymphocytes (no. of specimens and percentage) |              | Total no. of specimens |
|-------------------------|--|--------------|------------------------|
|                         | 50–100%<br>n (%)   | 50%<br>n (%) |                        |
| Serous or sero-mucinous | 31 (68%)   | 9 (41%)      | 40                     |
| Mucoid                  | 48 (64%)   | 12 (35%)     | 60                     |

The figures in parentheses are the mean percentages of ANAE-positive cells.

vertically uniform. There was a decrease in the density of AChE staining within the subplate layer (Fig. 4).

In oldest fetuses examined (28 weeks of gestation) a pattern of vertical darkly stained alternating zones (140–200  $\mu\text{m}$  wide) appeared within the AChE-positive middle third of the cortical plate (Fig. 5 arrows). These vertical positive zones (columns) are separated by narrower interspaces of lower staining density. AChE-positive columnar zones could be followed in serial sections in a rostrocaudal direction.

## DISCUSSION

The observation presented in this paper provides evidence that there is a characteristic distribution and evolution of AChE staining within cytoarchitectonic compartments of the developing human auditory cortex. The early bilaminar pattern of AChE staining (marginal zone and subplate layer) corresponds to the bilaminar distribution of synapses (Molliver, Kostović & Van der Loos 1973; Kostović, Knežević, Kostović, Krmpotić, Nemanjić, Kelović & Vuković, 1978) observed in human fetal cortex. Thus AChE-positive fibres may be involved in early fetal synaptogenesis. The onset of AChE staining within the cortical plate at 24 weeks of gestation corresponds to the appearance of synapses in this layer (Molliver, Kostović & Van der Loos 1973; Kostović & Molliver 1974) and may indicate the establishment of thalamocortical circuitry. In accordance with this hypothesis is our finding of AChE-rich, vertically oriented alternating zones in prospective layer IV of the auditory cortex at 28 weeks of gestation. This columnar pattern of AChE staining which has been also observed by us in frontal cortex of human fetus (Kostović 1979) may correspond to the columnar distribution of thalamocortical fibres found in primate neocortex (Hubel & Wiesel 1969; Rakic 1976) or granule cells in primate auditory cortex (Smith & Moskowitz, 1979) while AChE-poor zones may correspond to the termination of cortico-cortical

fibres observed in the frontal cortex by Goldman & Nauta (1977). Vertically oriented AChE-positive bands may be also related to the columnar cortical organization identified by both physiological and neuroanatomical approaches (Mountcastle 1957; Hubel & Wiesel 1977; Hubel, Wiesel & Stryker 1978). Thus our observations (Kostović 1979; this study) may constitute the first evidence of columnar organization in the human cerebral cortex.

As suggested previously (Vaughan 1975; Krmpotić, Nemanjić, Kostović, Nemanjić & Kelović, 1979) the early establishment of the main organization of the auditory cortex may be of essential significance for both normal and abnormal development of the central auditory system.

At present we have no evidence that the AChE-positive reaction found in our material may reflect the cholinergic nature of the thalamic input to auditory cortex as was proposed for other sensory cortices (Kristit 1979). It is possible that the presence of AChE is related to the process of growth and differentiation and may be present in some developing telencephalic fibre systems (Kostović, Knežević, Kostović, Krmpotić, Nemanjić & Kelović, 1979; Kostović, Kelović, Kracun & Krmpotić, Nemanjić 1979). Furthermore the AChE-positive reaction may be related to some non-cholinergic inputs as suggested by Emson & Lindvall (1979).

## RÉSUMÉ

Dans cette étude les méthodes de coloration avec acétylcholinestérase (AChE) étaient adoptées pour démontrer le développement de fibres afférentes du fœtus cortex auditif dans les fœtus humains de 8 à 28 semaines de gestation. La première AChE positive réaction apparaît chez les fœtus de 20–4 semaines dans le neuropile de la zone marginale et dans le subplate layer du cortex auditif. Par cette coloration caractéristique avec AChE le cortex auditif peut être délimité des autres régions corticales. À 4–26 semaines pendant la lamination latérale de la zone corticale AChE-positive réaction fut trouvée dans la partie profonde de la zone corticale. Dans les stades de 28 semaines une structure en colonnades étroites et sombres dans le tiers interneuronal de la zone corticale fut observée. On peut en conclure que les fibres





Fig. 2. Similar analysis as in Fig. 1 but a more porous gel is used. The broad albumin band has shifted towards the anode. Serum references are on the left and right.

no protein bands that in this analysis would have proved different from those present in serum.

### *Immune Complex Disease*

Another line of research which is only now starting to gain importance is based on the proposition of Veltri & Sprinkle (1976) that the presence of a specific antibody—a source of antigen and lysosomal enzymes—could provide the basis for chronic immune complex damage to the middle ear mucosa. Mravec et al. (1978) tested this in an animal model injecting immune complexes into chinchillas' ears and found a typical complement-mediated acute inflammatory reaction. Recently Leimonen (1979) provided evidence that pneumococcal capsular antigens are almost invariably present (88%) in the middle ear liquids from which pneumococci have been cultured. In addition these antigens were present in 33% of the non-specific pathogen liquids in which the cultured agent was *Hemophilus influenzae*. In some liquids the lack of antigens was

apparently due to the fact that the antigens were already fixed in immune complexes. Indeed, they could be separated by heating; pneumococcal antigen becoming denaturable.

### COMMENT

Presently two new aspects of immunologic mechanism apparently pertaining to the chronicity of secretory otitis media seem to emerge. One is the normal frequency of T cells in both the serous and mucoid cell-line effusions, which as such is evidence of an adequate T-cell response. However, these cells are associated with the development of delayed-type hypersensitivity reactions which arise from local contact with suitable antigens. Two main antigen groups may here be of importance: viz. food antigens with which the middle ear mucosa comes into contact during the bottle-feeding period, and bacterial antigens which attack the mucosa at any age. The importance of this mechanism is still a matter for future research.

Tabelle II:  $^3\text{H}$  Thymidin Markierungsindex und die histopathologischen Angaben der Larynxkarzinome 1974-1978

|  | Differenzierungsgrad |                               |  |
|--|----------------------|-------------------------------|--|
|  | Hochdifferenziert    | Mittelmäßig (moderately) diff | Gering, differenziert und undifferenzierte |
| Grades                                 | 79                   | 31                            | 18   |
| $^3\text{H}$ Markierungsindex, L.I. %  | (5-7,8) 6,5          | (7-10,5) 8,5                  | (9,8-15,1) 11,9                            |
| Markierungsindex, M.I. %               | (0-7,3) 1,08         | (0,9-1,1) 1,03                | (1,5-4,1) 2,9                              |
| Zellkern-Morphologie (nuclear grading) | NG 3                 | NG 3-NG                       | NG 1                                       |
| Nekrosen                               | 0 +                  | Gering-bef (irregular)        | Tief infiltrativ (highly)                  |
| Invasion (in der Randzone)             | Ungrenzt             |                               |  |

Es sei betont, dass der M.I. inkubierten Gewebes immer geringer als in vivo-Verhält. Dieses ist weil die im Gang befindlichen Mitosen während der Inkubation ablaufen aber neue Zellen aus der G<sub>2</sub>-Phase in die M Phase nicht übertreten (Rayewsky Oehlert).

## ERGEBNISSE

Auf Grund des histologischen Differenzierungsgrades sind die Kehlkopfpatienten in 3 Gruppen eingeteilt worden

- in der I Gruppe: Kranken mit hochdifferenzierten,
- in der II Gruppe: Kranken mit mittelmäßig differenzierten
- in der III Gruppe: Kranken mit gering differenzierten Karzinomen.

Dem Reifegrad entsprechend wurden auch die Zell- und populationskinetischen Parameter gruppiert.

Die Tabelle I zeigt die mit in vivo und in vitro  $^3\text{H}$ [TdR] Markierung bestimmten und geschätzten Werte

Die Werte der Markierungsindices sind innerhalb einer Gruppe auch nicht gleich trotzdem kongruieren sie mit dem Differenzierungsgrad des Tumorgewebes gut.

Die Wachstumsfraktion (G.F.) erweist sich als eine andere charakteristische Angabe. Mit Abnahme der Ausbreitung der Tumorzellen steigt die Zahl der proliferierenden Zellen

des Gewebes. Es entspricht der von Stein abgefasster Grundregel mit der Abnahme der strukturellen Differenzierung nimmt die funktionelle Kapazität ab und es steigt die reproduktive proliferative Kapazität des Gewebes.

Der Zellverlust beträgt mehr als 90% stammt aus Zelltod Migration und die Exfoliation spielt bei den Larynx Tumoren eine wesentliche Rolle.

Die nach in vitro  $^3\text{H}$ [TdR] Incorporation bestimmten Angaben von 78 L.I. wurden nach dem strukturellen Differenzierungsgrad gruppiert. Die Ergebnisse sind in der II Tabelle dargestellt.

Die Werte der Markierungsindices und mit dem histopathologischen Befunden in gutem Einklang und ändern sich zwischen 5-15%. Ein L.I. der unter 8% liegt ist für die gut differenzierten Geschwülsten charakteristisch. Der L.I. wurde nur dann über 10% gefunden wenn das Karzinom geringdifferenziert oder undifferenziert war.

Mit den anderen morphologischen Kennzeichen der Malignität des Tumors zeigte der L.I. eine gute Korrelation.

Die Zellkernatypie ist bei den hochdifferenzierten Geschwülsten nach Bloom und Black (NG3) geringer aber mit der Abnahme des strukturellen Reifegrades können sowohl in Form, als auch in der Größe und Färbung bedeutende Unterschiede gefunden werden (Pleomorphismus NG1 NG1).

Die Ausdehnung der Nekrose bei nicht

## PROBLEMS CAUSED BY IMMUNODEFICIENCIES

J Jákó

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The identification and functional classification of the various immunodeficiencies do not seem to provide the final answer to the problem. From the aspect of didactics as to its tasks and function the immunoapparatus can be divided into three major areas: the afferent branch, the central portion and the efferent branch.

I shall treat my subject according to this division.

The afferent branch which takes care of antigen uptake is split into two phases: one aspecific and one specific. The aspecific phase includes the activity of the segmented leukocytes. Should it deviate from the normal, the patient, as in any other deficiency of the immunoapparatus or of other areas, will suffer from a persistent sino-broncho-pulmonary infection. But for the clinician the picture is by no means unambiguous. He recognizes the deficiency of resistance but the clarification of the basic pathological process calls for great efforts and considerable instrumentation. The deficiencies of the leukocytes are mostly genetically determined.

Whether the organic reaction to antigen stimulus is cellular or humoral depends on the specific phase.

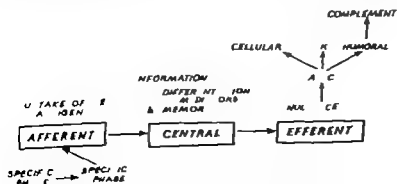
The first possibility is that the number of epitopes in the antigen is very small and if they attach to the cell surface, there will be no more free epitopes and no interaction between the T and B cells. The result will be a cellular immunoresponse.

In the second consideration is the number of epitopes in the antigen greater. A cell-to-cell interaction will take place since one of the epitopes attaches to the T and the other to the B cell producing a humoral antibody response.

The third possibility is the presence of an antigen with a large number of epitopes in a low concentration which, considering the probability of their linking, is incapable of linking T and B. The outcome, once again, is a cellular immunoresponse—the phenomenon of low zone tolerance.

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## PROBLEMS CAUSED BY IMMUNODEFICIENCIES

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The identification and functional classification of the various immunodeficiencies do not seem to provide the final answer to the problem. From the aspect of didactics as to its tasks and function the immunoapparatus can be divided into three major areas: the afferent branch, the central portion and the efferent branch.

I shall treat my subject according to this division.

The afferent branch which takes care of antigen uptake is split into two phases: one aspecific and one specific. The aspecific phase includes the activity of the segmented leukocytes. Should it deviate from the normal, the patient—as in any other deficiency of the immunoapparatus or of other areas—will suffer from a persistent sino-broncho-pulmonary infection. But for the clinician the picture is by no means unambiguous. He recognizes the deficiency of resistance but the clarification of the basic pathological process calls for great efforts and considerable instrumentation. The deficiencies of the leukocytes are mostly genetically determined.

Whether the organic reaction to antigen stimulus is cellular or humoral depends on the specific phase.

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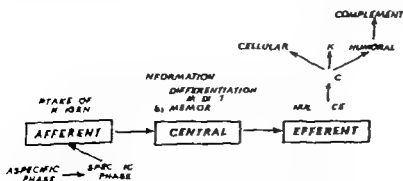


Fig. 1

## STREPTOMYCIN INDUCED DEFECTS OF THE OTOCONIAL MEMBRANE

Lars-Göran Johnsson, Charles G. Wright, Robert E. Preston and Pamela J. Henry

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**Abstract.** Damage to the neuro-epithelium caused by the ototoxic drugs has been well described in previous reports, however little is known about the effect of such drugs on the otoconia. In this investigation streptomycin sulfate is found to create a circumscribed defect in the crystalline layer of the otoconial membrane in the utricle of the guinea pig. The defect was secondary to a sharply outlined lesion of the neuroepithelium which corresponded in size and location to the otoconial defect. Many of the otoconia along the margin of the defect showed signs of degeneration and appeared similar to those observed in the saccule of man during aging. In advanced stages of decay the otoconia were reduced to hollow shells consisting of longitudinal interconnecting strands. All the epithelial lesions occurred with remarkable consistency in the same region of the macula utriculi at the posterior end of the stria.

The aminoglycosides streptomycin, gentamicin, and tobramycin are the most important vestibulotoxic drugs. Their major site of action is the neuro-epithelium and the damage they cause has been well documented (Hawkins 1976). This report deals with injury to the utricular otoconia caused by streptomycin.

After intratympanic application of streptomycin, the sensory epithelium of the macula utriculi shows less damage than the ampullary cristae and the macula sacculi still less (Lundman 1969). It is of interest to note that the Type I hair cells located within the striola (Wersör 1940) are more vulnerable than the Type II cells. Streptomycin is also known to affect the so-called dark cells in the membranous wall of the utricle (Hawkins & Preston, 1973).

Reports of otoconial abnormalities have been few. We have observed what probably

are defects in the otoconial membrane in guinea pigs after gentamicin (Hawkins et al 1969) and neomycin (Johnsson & Hawkins 1972) treatment. Neomycin appeared to cause loss of otoconia on the posterior tip of the saccule while gentamicin created more generalized loss of saccular otoconia with some giant otoconia remaining. Because the specimens had been stored for several months in 70% alcohol the pH of which was not measured the validity of these observations is open to question. Lim (1973) has observed abnormal otoconia in the guinea pig which he believes were altered due to ethacrynic acid treatment. Recently loss of both saccular and utricular otoconia has been observed in guinea pigs after administration of streptomycin (Harada & Sugimoto 1977). As has been mentioned in our preceding report experimentally induced otoconial abnormalities in guinea pigs have to be viewed in light of the fact that defective otoconial membranes do occur in untreated control animals (Johnsson et al 1979).

This report deals with an otoconial defect which with certainty can be related to degenerative changes in the neuro-epithelium. The observation was made during dissection of the vestibular system in guinea pigs intended for use in a study of the effect of streptomycin on the incorporation of  $^{45}\text{Ca}$  in the otoconial membranes.

## METHODS

The technique of microdissection which was employed in the previous study (Johnsson et al 1979) was also used in this investigation.

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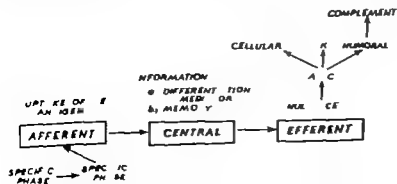


FIG. 1

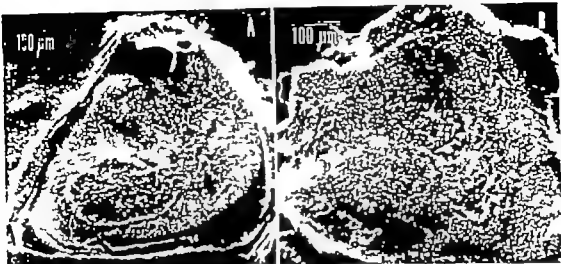


Fig. 3. Scanning electron micrographs of the right utricular maculae from 2 animals killed 30 days after treatment. The tufts of sensory cells are displayed. The posterior tip of the macula is in the lower left and right corners

respectively. A, Single large lesion. The margin of the defect is thickened (critical arrow). B, Small defect (horizontal arrow). Not the sensory cell loss along the striola.

clearly accentuated the original defect in the otoconial membrane.

### DISCUSSION

This study has demonstrated a more or less precise local correlation between a defect in the crystalline layer of the otoconial membrane and a streptomycin-induced lesion in the neuro-epithelium of the macula utriculi in guinea pigs. One can only speculate about the process which caused the loss of otoconia. It is highly improbable that purely mechanical factors were involved. We believe it is more likely that the epithelial damage interfered in some way with the calcium metabolism of the otoconia. Recent studies (Preston *et al.* 1975; Mechligan *et al.* 1979) have provided evidence for active turnover of calcium in the otoconial membranes. Thus the streptomycin treatment may have disturbed biochemical mechanisms responsible for calcium transport or uptake. Also during degeneration of the neuro-epithelium, lysis of cells probably occurred leading to the release of lysosomal products and enzymes which may have low

ered the pH sufficiently to cause dissolution of the otoconia. Such products of cellular degeneration might in addition alter the organic matrix of the otoconia as well as the gelatinous portion of the otoconial membrane.

Our findings show little similarity to the otoconial changes recently attributed to streptomycin treatment by Harada & Sugamoto (1977). The degenerating otoconia in our specimens did appear similar to those found in the human sacculus during aging (Ross *et al.* 1976) which suggests that a common process might be involved in production of the two seemingly different forms of otoconial degeneration.

This study does not show whether loss of sensory cells and supporting elements alone unaccompanied by focal defects of the maculae can alter the otoconia. It seems likely that this will occur with time (Mechligan *et al.* 1979).

The marked loss of Type I sensory cells in the utricle was to be expected (Lindeman 1969) but the circumscribed lesion occurring consistently at the posterior end of the striola was not anticipated. This finding suggests the presence in the utricle of a *locus minoris resis-*





glass in inverted-tip type electrodes. Because a 1 mV error in the reading is only equivalent to a Na<sup>+</sup> concentration change of 0.04 mM at the endolymph concentration, spatial separation of the tips of the electrodes was not a serious problem. In a subsidiary series of experiments, single pH microelectrodes of the recessed-tip type (tip diameter 2.5 µm) made from H<sup>+</sup>-sensitive glass (Corning 0150) were used in place of the Na<sup>+</sup>-sensitive electrodes.

Repeated measurements of the calibrating solutions always gave identical readings and a better estimate of the sensitivity of the method was therefore required. All potentials were recorded to  $\pm 50$  µV. For the Na<sup>+</sup>-sensitive microelectrode this was equivalent to  $\pm 0.002$  mM at the 1 mM level and  $\pm 0.07$  mM at the 10 mM level. The corresponding figures for K<sup>+</sup> were  $\pm 0.28$  mM at 150 mM and for pH  $\pm 0.001$  unit at pH 7.4. The results are volumetrically expressed in the form: mean  $\pm$  standard error of mean (number of experiments) (unless this is given in the adjacent text). The values give ionic concentrations and not activities to enable comparison with other findings to be made more easily.

#### Permeability and flux calculations

Due to the absence of a suitable direct method of permeability alterations were quantified by means of the principle introduced by Hodgkin & Katz (1949) and developed for the cochlea by Johnstone and his colleagues (Johnstone 1970; Sellick & Bock 1974; Sellick & Johnstone 1975). In essence the rate of change in the endolymphatic concentration of the ion concerned for any selected moment during terminal anoxia, normalised with respect to the relevant electrochemical gradient is used to calculate the conductance of the ion at that moment according to the equation

$$\lambda G = \frac{\Delta I}{\frac{RT}{zF} \ln \frac{a'}{a} + \Delta E}$$

where  $R$  is the gas constant,  $T$  is the absolute

temperature,  $z$  is the valency of the ion,  $F$  is Faraday's constant,  $a'$  and  $a$  are the activities of the ion in endolymph and perilymph respectively,  $\Delta E$  is the electrical potential difference between endolymph and perilymph,  $\Delta I$  is the rate of change in the endolymphatic concentration of the ion,  $G$  is the true conductance and  $\lambda$  is a constant. Although  $\lambda$  is specific for any particular animal species, its value is not known precisely and the term  $\lambda G$  is taken as an index of the permeability of the ion which enables relative permeabilities and permeability variations to be determined accurately. In anoxia its value remains unchanged for 3–6 min after the minimum peak in the endocochlear potential following which substantial increases in cation permeability occur. The cationic conductances during this short stable period have been shown to be representative of the pre-anoxic conditions (Bosher 1979).

When multiplied by the appropriate ionic driving force, conductances determined in this way provide an estimate of the rate of concentration change due to the passive forces at any time during the experiment. Thus the contribution of the active transport processes can be derived from the net concentration change according to the equations

$$cJ_{\text{pass}} = \lambda G_i \left( RT \ln \frac{a'}{a} + \frac{EP}{F} \right)$$

$$cJ_{\text{act}} = cJ_{\text{net}} - cJ_{\text{pass}}$$

where  $J_{\text{net}}$ ,  $J_{\text{act}}$  and  $J_{\text{pass}}$  are the active, net and passive ionic fluxes at time  $t$ ,  $c$  is the volume area constant for the endolymph system of the animal (so  $cJ$  is the rate of concentration change) and  $EP$  is the endocochlear potential. While the true fluxes ( $J$ ) cannot be specified because  $c$  has not been measured with sufficient reliability, the results do enable the various ionic fluxes to be compared with each other and the changes in the fluxes with time can be followed.

DEFICIENCY OF  $\text{C}_3$   
 DEFICIENCY OF  $\text{C}_2$   
 CONGENITAL HYPERCATABOLISM OF  $\text{C}_3$   
 FAMILIAL DYSFUNCTION OF  $\text{C}_2$

Fig 9

All congenital anomalies caused by lack or dysfunction of the individual complement factors will manifest themselves in a series of infections

Summing up In the time allocated and due to the large volume of information it was impossible to go into greater detail on this extremely interesting subject I have attempted to give a survey of the functioning of the immunoapparatus from the aspect of the antibody deficiency syndromes However I would like to call attention to the fact—which follows unambiguously from this lecture—that if the case in hand is suspected of immunodeficiency not only must the immunoapparatus be examined but very often also the macrophages the leukocytes and the complement system Fortunately genuine immunodeficiencies are few and far between but the clinician is often confronted with pathological processes which differ from the commonplace Some transitions may exist which are not immunodeficiencies merely a weakness of the immunoapparatus and should this happen the physician may further aggravate the situation for instance by antibiotic therapy

### RÉSUMÉ

La brièveté de temps et la multitude des données diverses ne m'ont pas permis de m'approfondir dans les détails

extrêmement intéressantes Le but que j'ai poursuivi embrasser d'un coup d'oeil la fonction de l'appareil immunologique sous l'angle des symptômes déficients des trois corps J'attire l'attention ce que d'ailleurs ressort de tement de mon cours a ce que si le soupçon d'une déficience immunologique subsiste il s'impose non seule la prospection dans le sens étroit du mot de l'appareil immunologique mais souvent l'examen des globules blancs des macrophages et du système complémentaire Heureusement la vraie déficience immunologique est malade des plus rares mais le clinicien constate souvent un aspect pathologique différent du processus habituel Probablement ils existent des transitions ou il ne s'agit de déficiences immunologiques mais des simples faiblesses et dans ce cas d'une façon inconnue le médecin joue comme facteur affaiblissant un certain rôle par suite d'une thérapie antibiotique

### ZUSAMMENFASSUNG

Die Zeit, die mir zur Verfügung stand sowie die vielfältig verzweigten Angaben haben es ermöglicht, was in einzelnen außerordentlich interessanten Detailfragen vertiefen Mein Ziel war über die Funktion des Immunapparates vom Gesichtspunkt der Syndromen mit Immundefizienz (Antikörpermangel) einen Überblick zu geben Ich möchte die Aufmerksamkeit darauf lenken, folgt aus meinem Referat eindeutig — daß im Falle Verdachtes auf ein Krankheitsbild von Immundefizienz nicht nur der Immunapparat selbst sondern oft auch Leukozyten die Makrophagen und auch das Komplementsystem untersucht werden müssen Glücklicherweise ist die echte Immundefizienz eine außerordentlich seltene Krankheit Viel öfter sieht der Kliniker ein Bild, das gewöhnlichen Krankheitsablauf abweicht Höchstens scheinlich gibt es solche Übergänge bei denen es nicht um die Immundefizienz, sondern nur um eine Schwäche handelt In diesen Fällen kann auch der Arzt auf bisher unbekannte Weise — z.B. mit der Therapie durch Antibiotika als ein weiterer erschwerender Faktor mitwirken

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as in the case of Na<sup>+</sup> but faster in the case of K<sup>+</sup>. As described below these findings (Fig. 7) indicate that ethacrynic acid given intravenously has essentially the same actions in the two species, the differences found being only of a minor quantitative nature.

#### Permeability alterations

Each experiment was terminated with anoxia induced at varying times after the initial injection and the relative alterations in the permeabilities of the endolymphatic membranes which had arisen were calculated as described earlier. The results (Fig. 3) showed that the ethacrynic acid had produced decreases of  $40(\pm 4)\%$  in the Na<sup>+</sup> permeability ( $n=10$ ) and  $77(\pm 3)\%$  in the K<sup>+</sup> permeability ( $n=9$ ) in the rat. These permeability effects were present at 90 min and were long-lasting, no evidence of recovery being present at 120 min. Attempts to determine the time course of the reductions more precisely were unsuccessful. This was because some ethacrynic acid probably remained in the inner ear tissues when anoxia was induced shortly after the drug administration. In these circumstances the permeability changes would eventually develop fully during the anoxic period due to its continued action. It will be noted that these permeability effects proved to be different in type to those expected from our preliminary investigation (Bosher et al. 1973). An advantage of the dose of ethacrynic acid selected was that the rise in the endolymphatic Na<sup>+</sup> concentration was well below the level of 12 mM. This is the concentration which is associated with a marked increase in the permeability of the endolymphatic membranes to cations (Bosher, 1979). The complications of a secondary change of this type were therefore completely avoided. The guinea pig experiments were too few for the total range of the effects to be determined accurately. But the mean reductions found in the Na<sup>+</sup> and K<sup>+</sup> permeabilities were 45% and 68% respectively (i.e. about the same as in the rat).

#### Effect on active transport

The changes in the endolymphatic ion concentrations provide very little information by themselves about the effect of ethacrynic acid upon the active transport mechanisms. The marked alterations in the endocochlear potential produce equally marked alterations in the passive fluxes and these must be taken into account first. However this can be done as described earlier and the action upon the ion-transporting mechanisms is then revealed.

In every animal the active processes responsible for Na<sup>+</sup> and K<sup>+</sup> transport and endocochlear potential production were completely abolished by the time of the minimum peak in the potential. A problem arises about the exact interpretation of the details of this action. As explained above it was not possible to determine experimentally the precise time courses at the beginning of the permeability effects. So the initial time courses illustrated in Fig. 3 were used in the calculations. Although these were undoubtedly good first approximations the early differences between the various processes apparent in Fig. 4 might have been due to small variations in the true time courses of the permeability effects rather than actual divergences between the processes themselves. For example the initial small rise in Na<sup>+</sup> activation might have been due to a slight transient increase in Na<sup>+</sup> permeability before it declined to its later level (Fig. 3). Whatever their cause these initial differences were relatively minor in nature and activation was reduced to zero at the time of the minimum endocochlear potential in every case. Consequently the principle effect of the ethacrynic acid in this respect was to fully inhibit all the transport processes measured at about the same overall rate.

At and after the time of complete inhibition the reductions in permeability measured individually could be used in each experiment. Thus similar problems do not arise about the findings in this period. These revealed that the Na<sup>+</sup> transport increased much more quickly

cytes was investigated in repeatedly inflamed human tonsils simultaneous with the study of their morphologic structure

## MATERIAL AND METHODS

### *Patients*

20 patients (10 male and 10 female average age 19.4 and 8.9 range 3-44 and 3-24 years respectively) were tonsillectomized because of repeated tonsillitis. Tonsils of 3 dogs were used for comparison.

### *Lymphocyte activation test*

Immediately after excision the tonsils were placed into ice-cold 0.9% saline soon after (30 min) a cell suspension was prepared by mechanical disaggregation of the tonsils in a culture medium (RPMI 1640, Difco). The cells were counted in a haemocytometer and  $1 \times 10^5$  viable cells in 0.01 ml culture medium were dropped on glass slides dried and stained with nivalol according to Kellermayer & Jobst (1970) and a polarization optical analysis was performed (Surján & Sebők 1973). The frequency of nuclear birefringence of tonsillar lymphocytes was determined and the presence of activated cells with increased birefringence was sought in addition to the normally distributed non activated cell population. The differences between the observed distribution and the normal distribution were analysed by means of the  $\chi$ -square test.

### *Light and electron microscopy*

The tonsils were fixed in a phosphate buffered (pH 7.2) 4% formaldehyde solution embedded in paraffin cut and stained with haematoxylin and eosin. In 5 cases the ultrastructure of the tonsils was also investigated. For that purpose the samples were cut into small pieces (1 cubic mm) fixed in 5% glutaraldehyde in phosphate buffer postfixed in a 1% osmium solution dehydrated and embedded in Araldite. Semithin sections stained with toluidine blue were prepared for selection of tissue blocks used in electromi-

croscopy. The thin sections were stained with uranyl acetate and lead citrate.

## RESULTS

**1 Structure of the reticular epithelium in inflamed human tonsils.** The tonsillar epithelium was invaded by lymphocytes, histiocytes, plasma cells, macrophages, granulocytes. These cells arrived at the superficial cells of the epithelium via interepithelial passages. While in healthy and the epithelial barrier between crypt lumen and lymphoid cells consists of a single layer of cells characterized by abundant intracellular tubulo-vesicular system (Fig. 3) in inflamed human tonsils the surface of reticular epithelium is covered by flat stratified epithelial cells and the M cells are found occasionally (Fig. 1). The intra-epithelial passages are usually covered with squamous epithelium (Fig. 2). The M cells if present have a reduced tubulo-vesicular transport system (Fig. 4). At some places the superficial epithelial cells contain lysosomes, myelin bodies or rough surface endoplasmic reticulum.

**2 Nuclear birefringence of unstimulated lymphocytes from human tonsils removed cause of recurrent inflammation.** The mean retardation and standard deviation of birefringent human tonsillar lymphocyte nuclei ( $18.37 \pm 4.3$  nm) are compatible with those of the animal tonsillar lymphocytes ( $16.33 \pm 2.5$  nm) (Surján 1977). The birefringent lymphocytes were however normally distributed in all but 3 cases ( $\chi$ -square test). The probability of occurrence of all cells having retardation equal to or greater than the mean  $+1.5$  S.D.

*Fig. 1* Semithin section from a repeatedly inflamed human tonsil. Only one follicle-associated M cell in the crypt epithelium  $\times 100$ .

*Fig. 2* Structure of metaplastic stratified squamous epithelium from repeatedly inflamed human tonsil  $\times 400$ .

*Fig. 3* Detail of a canine tonsillar M cell. Abundant tubules are present in the endoplasmic reticulum  $\times 1000$ .

*Fig. 4* M cell in repeatedly inflamed human tonsil. There are only few cytoplasmic vesicles  $\times 1000$ .

1979) However the location and extent of the biophysical component of the endolymphatic membranes responsible for the overall selective permeability has yet to be determined. A possible third component of the endocochlear potential has been described in the guinea pig (Sellick & Johnstone 1974 Kusakari & Thalmann 1976 Kusakari et al 1978) but has been shown to be completely absent in the rat (Bosher 1979). Consequently it does not complicate the interpretation of the experiments on this species reported here. In the 9 rat experiments in which simultaneous Na and K results were obtained the mean diffusion potential calculated from the standard Hodgkin & Katz (1949) equation and the measured values at the time of the minimum potential peak was  $-20.0 (\pm 4.4)$  mV. The average endocochlear potential actually found was  $-24.7 (\pm 3.9)$  mV the discrepancy of  $4.7 (\pm 0.9)$  mV being identical with the one described in other situations (Bosher 1979). The difference in the value of this negative peak to the one found after anoxia in normal animals is due to the decrease in the relative permeability of the endolymph system to K after ethacrynic acid the measured Na/K permeability ratio at this time being  $0.63 (\pm 0.08)$  compared with the normal 0.27 in the rat.

Finally the results also provide evidence about the relationship between the mechanisms responsible for cation transport. As reported, the normal ratio of active K to Na transport is 13:1 in the rat and 8:1 in the guinea pig. Although the latter value is less than the 66:1 derived theoretically for the guinea pig by Sellick & Johnstone (1975) it is still well outside the coupling ratios of the Na/K activated ATPases. In addition the large differences in the active transport systems demonstrated during the recovery period indicate that most of the K transport must be independent of the Na transport. These findings consequently provide further experimental evidence in support of the view that some enzyme other than a Na/K-activated

ATPase is directly responsible for the potential generation and at least most of the K transport (Kujpers & Bonting 1970b Sellick & Bock 1974 Sellick & Johnstone 1975 Paloheimo & Thalmann 1977 Kusakari et al 1978). It follows from this that the K transport will be largely and possibly completely electrogenic in nature and this is confirmed by the close association between the K transporting and potential-producing systems described in the recovery period.

The importance of Na/K-activated ATPase in the endolymph system has not been in question since the initial studies of the effects of ouabain (Konishi & Mendelsohn 1970 Kujpers & Bonting 1970a). Moreover it is present in the stria vascularis in a high concentration (Kujpers 1974 Kujpers & Bonting, 1969 Matschinsky & Thalmann 1970) but it cannot be concerned directly with endolymphatic cation transport. Instead preliminary results in this laboratory (Bosher unpublished observations) suggest its role to be an indirect one such as maintaining the internal ionic environment of the transporting cells.

## ACKNOWLEDGEMENT

I am grateful to the Department of Medical Illustration, The Middlesex Hospital for preparing the diagrams.

## ZUSAMMENFASSUNG

Endolymphatische Veränderungen infolge intravenöser Injektion von Ethacrynsäure an Ratten, 60 mg kg<sup>-1</sup> wog den bis zu 120 Minuten lang beobachtet unter Verwendung konventioneller und ionenselektiver (Na<sup>+</sup>, K<sup>+</sup> und pH) Mikroelektroden. Man fand als Ursache 3 deutliche Wirkungen auf das Endolymphsystem. Zunächst wurden die strukturelle potential-produzierenden und kationen-transportierenden Prozesse total gestoppt. Wiederherstellung begann schnell und war zunächst rapide. Dann verlangsamte sie sich erheblich, vermutlich infolge der Abnahme der strukturellen Energieproduktion mit langsamem Beginn und langer Dauer. Gleichzeitig mit diesen Veränderungen der gesamten Kationenpermeabilität des Endolymphsystems auf Dauer hatte einen verschiedenartigen Zeitablauf und betraf K<sup>+</sup> erheblich mehr als Na<sup>+</sup>. Die Befunde gaben außerdem weitere Informationen über den Mechanismus, der für die normale Beschaffenheit

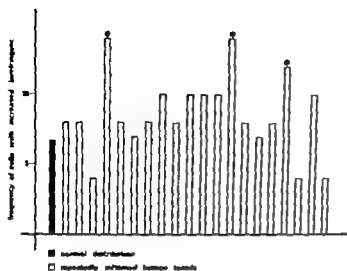


Fig 5 Frequency of the cells having equal or greater nuclear birefringence than the mean  $\pm 1.5$  S D. Statistically significant differences from the normal distribution were found only in 3 cases (asterisks)

6.68% under conditions of normal distribution. The occurrence of such cells in the 20 human tonsils is demonstrated in Fig 5. In 3 cases these cells with high nuclear birefringence were present in 14, 12 and 14% and the difference from the normal distribution is statistically significant with a probability of 0.001, 0.05 and 0.01 respectively.

**3 Histology of tonsils with and without lymphocytes of increased nuclear birefringence.** Histology of the three tonsils with increased nuclear birefringence (patient age 3, 3 and 6 years respectively) was contrasted with that of tonsils containing normally distributed birefringent lymphocytes. Tonsils containing activated lymphocytes have a faucal surface epithelium which is not (or is only slightly) infiltrated with lymphoid cells. The subepithelial connective tissue is also almost free from lymphoid infiltration (Fig 6). By contrast the surface epithelium of the other tonsils is infiltrated by lymphocytes and thus this epithelium reminds one of crypt epithelium (Fig 7). The crypt epithelium of the three activated tonsils is highly reticular and the epithelial barrier between crypt lumen and lymphocytes is narrow, usually consisting of only one cell layer (Fig 8). The crypts of

the other tonsils are lined by a stratified and in some places keratinized epithelium (Fig 9) and M cells are seen only exceptionally. Fibrosis of varying degrees was observed in the inflamed tonsils even in young children. The lymphocyte mantle of follicles usually reached the reticular epithelium but in some inflamed tonsils a wide extrafollicular lymphoid tissue is interposed (Fig 1).

## DISCUSSION

The lack of appropriate controls is the main difficulty in the study of human tonsils. Animal tonsils are useful merely for comparison purposes. The value of autopsy material is questionable since child mortality is happily low and adequate control tonsils are hardly available. Furthermore the disease of the patient might affect the tonsillar structure and/or function. The use of tonsils obtained from forensic dissections may be an alternative solution of the problem but the post mortem changes also raise difficulties for the investigation of structural or functional details. The best is the study of tonsillar biopsies from healthy volunteers but this has certain limitations too: the small volume of biopsy material is perhaps not perfectly representative of the whole tonsil.

While researchers without appropriate controls are unable to develop diagnostic methods for tonsillar diseases and there is a continuing debate over the effectiveness of tonsillectomy, this operation is the most common surgical procedure. It is almost certain that not only diseased but also healthy tonsils are ectomized with unknown frequency. There are at present

Fig 6 Faucal surface epithelium of a human tonsil has no activated lymphocytes.  $\times 700$

Fig 7 Faucal surface epithelium infiltrated by lymphoid cells from a repeatedly inflamed human tonsil.  $\times 700$

Fig 8 Reticular crypt epithelium from a human tonsil containing activated lymphocytes.  $\times 300$

Fig 9 Crypt epithelium from a repeatedly inflamed human tonsil. There is stratified non-reticulated squamous epithelium on the surface.  $\times 300$ .

## SPEECH DISCRIMINATION OF PATIENTS WITH HIGH FREQUENCY HEARING LOSS

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**Abstract.** Mean speech discrimination curves were measured in three groups of patients having severe, moderate and high-tone hearing losses in pure-tone audiograms, 20 patients being tested in each group. The audiograms sloped linearly by 22 dB/oct. Recruiting ears in the test were excluded. The group with moderate hearing loss achieved significantly poorer discrimination than at high sound pressure levels than the group with the hearing loss. A phenomenon not reported before, however, the results agree with preceding work in which mild sensorineural hearing losses were simulated by filter equipment. The causes of the phenomenon and significance for hearing aid selection are discussed.

$2 \times 10^{-3} \text{ N/m}^2$  and 75% discrimination. This phenomenon has not been reported before in the literature and it encouraged the author to attempt a measurement of the discrimination curves of patients having an equivalent clinical high frequency hearing loss. A further indication for this study was the lack of earlier results concerning the discrimination ability of Finnish-speaking patients with various grades of high frequency hearing loss.

clinical experience has often given the impression that some patients with relative serious high-pass hearing loss can utilize the remnants of their hearing better than some with moderate loss; but in other respects similar hearing loss as in an earlier experiment made to examine this phenomenon, three degrees of low-pass hearing loss were simulated using a filter apparatus and the corresponding discrimination curves measured using young subjects with normal hearing (Klukaanniemi 1979a). It was shown that theoretically there really are some significantly disadvantageous high frequency hearing losses which may yield markedly poorer discrimination scores at high sound pressure levels than other more serious hearing losses of the same type.

One case of this kind proved to be a linearly increasing hearing loss of 22 dB/octave beginning at about 250 Hz. The discrimination curve for this hearing loss crossed the curve yielded by a parallel hearing loss beginning at 25 Hz. The crossover point was 84 dB re

## MATERIAL AND METHODS

72 patients whose pure tone audiogram showed typical high frequency hearing loss were selected for further examination. An anamnesis was taken in order to outline 1) etiology of the hearing loss, 2) use of a hearing aid or other auxiliary facilities, 3) the patient's subjective opinion on his/her difficulties in daily life.

The ear status and pure tone threshold of the patients were examined and those showing recruitment in Reger's test were excluded so as to achieve a loudness function and its influence on the threshold curve equal to the preceding experimental study. The elimination of recruiting ears may increase the relative number of retrocochlear losses in the present material. The preceding experimental study with filtered words using normal-hearing subjects gave a reason to anticipate a very disadvantageous high frequency hearing loss among non-recruiting ears. Discharging ears were similarly eliminated from the subsequent tests.



no worldwide accepted criteria to sort out the healthy tonsils from the diseased ones

Earlier publications suggest that histology is suitable for this kind of selection. Eighty tonsils with relatively slight or without any noteworthy lesions in the histopathological appearance were selected from 165 cases of tonsillectomy under the diagnosis of the enlarged tonsils or chronic tonsillitis by Awaya (1969). From 107 cases of clinically chronic tonsillitis 96 have shown the histologic features of chronic tonsillitis in preoperative biopsies (Papuraw Malewa & Daskalow 1972). According to the latter authors the reticulation of crypt epithelium is not normal but suggestive of inflammation. However the reticulation is a physiological phenomenon in tonsillar crypts (Falk & Mootz 1973). The histologic criteria of chronic tonsillitis were never described and the value of these criteria has never been proved in comparison with normal controls. Weibel (1965) did not detect any significant abnormality except some fungal infection: his pathohistological study consisted of 4 680 patients. According to his suggestion routine histological examination of tonsils should be done only after the age of forty. Others have found malignant diseases of tonsils which could not otherwise be diagnosed and treated in time except by systematic histologic studies (Nicoucar & Schrago 1969; Sodagar & Mohalatee 1972). Considering that histology is not suitable for the objective testing of chronic tonsillitis other methods were suggested for the characterization of clinical stages of tonsils (Sprekelsen 1975; Siegel 1978).

The results presented here suggest that a distinction of healthy tonsils from chronically inflamed ones might be possible. Our material was divided into two groups: one with a normally distributed birefringent lymphocyte population and the other containing an additional group of lymphocytes with increased nuclear birefringence. Lymphocyte nuclear birefringence increases after phytohaemagglutinin (Surján & Sebók 1973) and antigen (Surján 1977) stimulation. This increase in the bire-

fringence is thought to be mediated by a sol factor of lymphocytes (Surján Juhász & Bók 1979).

Nuclear birefringence of lymphocytes only increases in the course of *in vitro* stimulation but it is constantly increased in pathological conditions connected with normal lymphocyte activation such as rheumatoid arthritis (Sebók, Talerman & Woth 1977). Another gut associated lymphoid organ the Peyer's patches contains lymphocytes with increased birefringence compared to lymph nodes or spleen (Surján & Kasz 1978). These data suggest that the increase in nuclear birefringence means activation of lymphocytes; hence the lack of lymphocytes with increased birefringence in all but 3 inflamed human tonsils suggested a reduced lymphocyte activation in the course of recurrent inflammation. The reality of this observation is supported by the histological investigations. Tonsils which contained lymphocytes with increased birefringence were similar to clinically normal tonsils (Brandtzaeg, Surján, Berdal 1978) and the other without increased nuclear birefringence were similar to the repeatedly inflamed tonsils which had reduced immunocyte density (Surján, Brandtzaeg, Berdal 1978).

The presented observations suggest a hypothesis: the metaplastic change in tonsillar crypt epithelium might be of importance in the pathogenesis of recurrent tonsillar infection. The repeated inflammations of tonsils produce a metaplastic crypt epithelium which contains fewer antigen receptor M cells than before which has stratified squamous cells: the luminal part of the epithelium and which is in some places not reticulated at all. Similar changes are well known in the adenoid where the difference of the original ciliated columnar epithelium from the metaplastic stratified squamous one is easily recognized. The metaplastic epithelium forms a barrier between the lumen and the lymphoid tissue and therefore the activation of the lymphocytes decreases and the number of Ig-containing cells

compared with that of the English speaker although there are large individual differences in speech spectra (Byrne 1977) the linguistic of the Finnish language may also add to the errors caused by upward masking of the second F in group C. Conversely however it also appears from the present results to be easier to utilize the remnants of one's hearing if one's audiogram belongs to groups B or D. Upward masking probably effectively eliminates the advantage which some Finnish patients (group C) with high frequency hearing loss have due to the fact that the speech power in their language is concentrated in the frequency area where their residual power of hearing lies.

The present results and those of the preceding experimental study suggest that the sloping frequency area where the disadvantageous masking effect is present is rather narrow about 1 octave. Nevertheless mild high frequency hearing losses are frequently located precisely in this area. These facts make it important in clinical practice to single out patients belonging to group C when selecting a hearing aid. The distorting masking noise of the first formant is best eliminated by a hearing aid in which amplification starts at 500 Hz. As seen here the patients in this group subjectively chose such a hearing device because of its significantly better discrimination ability. Another possibility would be a device which would present the main frequency areas of F<sub>1</sub> and F<sub>2</sub> to opposite ears. Some patients however show a third type of masking, central masking (Denaberg & Pickett, 1974) which eliminates even the advantage of this kind of hearing aid. Also the exact separation is difficult, because F<sub>1</sub> and F<sub>2</sub> very often overlap.

### ZUSAMMENFASSUNG

Sprachverstehensklassen es für drei Gruppen von Patienten mit einem schweren, mittelschweren und leichten Hörförder wurden mit den finnischen PB-Wörtern getestet. 20 Patienten werden in jeder Gruppe untersucht. Das Audiogramm jedes Patienten zeigt parallel 22 dB/Oktave. Der Unterschied zwischen dem positiven Reizniveau in Reiger Tests und dem unteren Schwellenwert. Die Gruppe mit dem mittelschweren Hörverlust erreichte bedeutend schlechteren Dis-

kriminationserfolg auf leichten Lautstärken als die Gruppe mit dem schweren Hörverlust — eine Erscheinung, die man nicht früher rapportieren hat. Die Resultate stimmen doch mit dem früheren Experiment überein, wo die äquivalenten sensorischen Hörverluste mit einem Filterapparat abgebaut wurden. Die Ursachen und die Bedeutung dieser Erscheinung für die Auswahl des Hörapparates wird diskutiert.

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Fig. 1. The macula of the saccule following ultrasonic irradiation towards the vestibule with 100 mw/20 min (H&E, 250 $\times$ ). Endolymphatic space *es*, neuroepithelium, *om* otolithic membrane.

1.5% veronal buffered osmium tetroxide (Bredberg et al. 1965). The results were compared with that occurring after a 4-week recovery period, assessed from conventional histological methods involving Heidenhain's iron fixation, nitrocellulose embedding, serial sectioning, and staining with haematoxylin and eosin.

## RESULTS OF VESTIBULAR IRRADIATION

Vestibular disturbances observed immediately after recovery from the anaesthesia included paralytic nystagmus accompanied by a head bobbing motion and loss of balance control with the cat falling onto its irradiated side. The severity of these effects was dependent on the intensity and duration of irradiation with the

strongest reaction following application of 100 milliwatts for 20 min. The direction of irradiation was found to be critical and was dependent on ultrasound being aimed towards the position of the ampulla of the superior semicircular canal. In most cases completely normal locomotory function was regained within ten days following the irradiation. These symptoms of balance dysfunction indicated vestibular irritation and animals not displaying this response were assumed to be incorrectly irradiated and were excluded from subsequent histological examination.

Four weeks after vestibular irradiation histological changes were observed within the vestibular neuroepithelium. Due to the variability of cellular changes it was impossible to relate the extent of damage to the ultrasound dosage and the following changes were observed following 100 milliwatts irradiation. In the saccule the otolithic membrane was often lifted away from the neuroepithelium (Fig. 1) causing damage to the hair cell cilia. The sensory epithelium lacked clarity of cellular detail and intercellular spaces occurred in this layer and in the underlying stroma. The basement membrane separating the epithelial layer from the stroma was not clearly differentiated. The otolithic membrane in the utricular macula was more resistant to damage but cellular disruption and cilia loss within the neuroepithelium was widespread. The cell walls were destroyed and the nuclei of the supporting cells and sensory cells clumped together into thin strands resulting in disorganisation of the normal stratified appearance of this epithelial layer (Fig. 2). Cellular detail in the underlying matrix was somewhat obscure.

Degenerative changes occurred to a lesser degree within the cristae in the ampullae of the semicircular canals. Deformities of the cupula commonly occurred in the superior ampulla, together with loss of cellular detail in the neuroepithelium. The extent of these changes was variable but there was a tendency for the utricular side of the crista to be more severely damaged than the semicircular canal side (Fig.

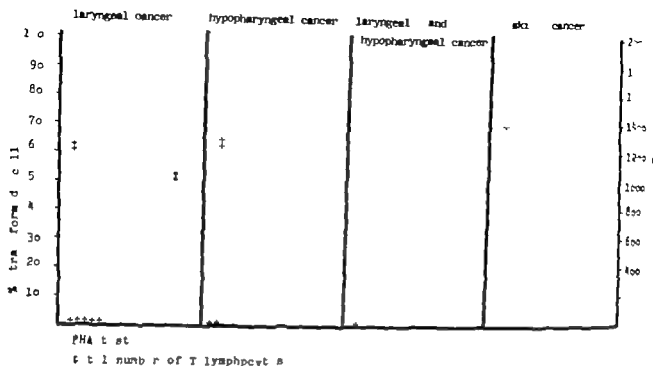


Fig. 1. PHA test and total number of T-lymphocytes in laryngeal, hypopharyngeal and skin cancer.

genic tumors bear antigens specific for the virus they crossreact with antigens of tumors caused in different organs of the same host but also with tumors caused in different individuals.

In 16 cases of laryngeal cancer we tested two tumor antigens which we obtained from squamous cell carcinoma and from cornuous squamous cell carcinoma—the two most common tumors of the larynx. Tumor antigens were prepared using a modification of the methods of Bloome *et al.* (1969) and Halliday (1972). We placed the freshly cleaned and minced tumor tissue in medium 199 (Institute of Immunology, Zagreb) which contained 100 IU of penicillin/ml and 60  $\mu$ g of streptomycin/ml. This was homogenized in a water bath in a Sorvall machine for 45 min at 1000 revolutions/min. The suspension was then transferred to a test tube and centrifuged at 1000 g for 30 min. The sediment obtained mainly contained membranes and larger subcellular particles. The supernatant was then centrifuged at 100,000 g for 60 min and the clear acellular supernatant was stored in 1 ml ampules at  $-20^{\circ}\text{C}$  until use.

Out of the 16 examinees with laryngeal cancer tested with tumor antigens 7 responded positively and 9 negatively. We considered a positive reaction to be cultures with more than 3% lymphocytes transformed by the antigen. Out of the 7 with positive findings, 2 died shortly thereafter, 4 are in good condition and 1 is in poor condition. The difference between the findings of the PHA test and the lymphocyte number in the two groups of patients was insignificant. Based on these results we cannot predict the outcome of the disease in laryngeal cancer by determining the *in vitro* reaction to tumor antigens. More than likely it will be necessary to determine the specific antigen for each patient. This represents a problem however considering the testing procedures involved and the expense of preparing these antigens.

#### HUMORAL AND CELLULAR IMMUNITY

It is considered that bone marrow and the fetal liver in man are the most likely sites for producing B lymphocytes. The majority of B lymphocytes



Fig 5 (A) A surface preparation of guinea pig cochlea showing the normal regular pattern of the outer hair cell cilia (Phase contrast, 1000). (B) Cochlear irradiation with 100 milliwatts resulted in shearing and distortion of the cilia, and some hair cell losses in the innermost row of the outer hair cell cilia (P.C., 1000). *hc* inner hair cells, *ohc* outer hair cells, *p* pillar cells.

round window ultrasonic treatment of Meniere's disease was sufficient to initiate pathological change.

Following vestibular irradiation changes in cell structure were observed in a restricted region of the basilar membrane proximal to the round window. The resulting impairment of cochlear function would involve the response to high frequencies above the range of what is considered to be practical hearing (Von Békésy 1960; Stevens et al 1935). The same ultrasound irradiation regime has produced no

deleterious change in the cochlear microphonic response to stimulus frequencies up to 8 kHz (Barnett 1979).

The results of cochlear irradiation demonstrated progressive cellular degeneration whereby the extent of immediate structural changes increased after a post irradiation period of latency during which cellular necrosis became histologically discernible. The immediate histological changes seem to be due to ultrasonically induced cellular agitation rather than a thermal mechanism as they have

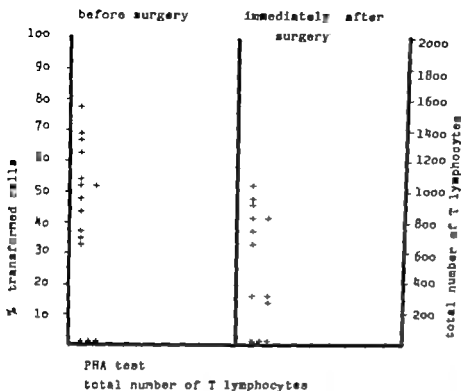


Fig. 3 PHA test and total number of T-lymphocytes following surgery

histologically examined the tumor and its surrounding tissue to check local lymphocytic infiltration. A positive correlation between the size of lymphocytic infiltrate and patient survival was found. The 1-5 year survival rate in patients with modest or good infiltration of lymphocytes was up to 70% but only 40% in those with small infiltration. The survival rate

was down to 25% in those with  $T_2$  tumors regardless of the type of treatment they received. If lymphocyte infiltration was small patients with larger  $T_2$  and  $T_4$  tumors the survival rates were over 50% in those with positive DNCB reactions and good infiltration up to 100% in those with positive PHA findings and strong infiltration. Sala & Fert

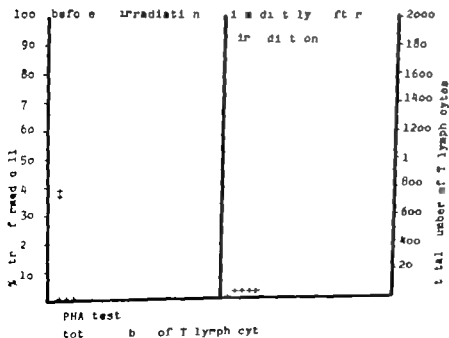


Fig. 4 PHA test and total number of T-lymphocytes following irradiation

## FATIGABILITY OF THE STAPEDIUS REFLEX IN INDUSTRIAL NOISE

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**Abstract** Normal hearing subjects were unilaterally exposed to 30 min of tape recorded shipyard noise (97 dB A) which is characterized by a variable temporal structure. The stapedius muscle activity was continuously recorded in the opposite ear as change of the ear's acoustic impedance. The reflex function was, in addition, assessed by stimulus-response curves obtained before and, at various times after exposure. A slight reflex fatigue was observed, together with a parallel shift of the stimulus-response curve (average 4 dB). The recovery was slow and not complete even 10 min after the end of the exposure. The individual variability was large. For 5 of the subjects the exposure was repeated at a later session and a individual degree of fatigue was found to be largely reproducible. The present results suggest that the stapedius reflex might play a role in the clinical picture of noise induced hearing loss.

Much interest has been focused on the stability and decay of the stapedius reflex response to constant pure tone stimuli and noise signals (Kato 1913 Wernick 1958 Dailon 1964 Hughes and et al 1966 Johansson et al 1967 Metz 1968 Coles, 1969 Kaplan et al 1976 Jämsen and Martin, 1978 Wilson et al. 1978). These studies all show very rapid adaptation of the response. It is however also known that a reflex response which has decayed will reactivate after even a very short pause (Metz, 1951 Wernick 1958 Borg & Ödman 1979) or after a change in intensity or frequency (Gävernes & Sjöboel 1966). The sound usually resulting in damage to the inner ear—industrial noise—varies with respect to frequency and intensity. It is therefore difficult to predict from available data how the stapedius muscle acts during a workday in an industrial noise environment and thereby the possible role of

the reflex in noise induced hearing loss. The presently prevailing opinion is that the muscle rapidly adapts (e.g. Tonndorf 1976) but there are also some early observations (Lüscher 1930 Kotrak et al. 1941) which indicate that this might not be the case. Lüscher observed the stapedius tendon through a perforation in the ear drum. He described a rapid adaptation upon continuous steady sound stimulation but a remarkably great resistance to fatigue in a more varied sound environment.

The aim of the present work was to study the stapedius reflex characteristics in a realistic industrial noise environment. Normal hearing subjects have been unilaterally exposed under laboratory conditions to a 30 min sequence of noise recorded in a shipyard. The stapedius reflex was continuously recorded in the contralateral ear and stimulus-response curves at 0.5 and 2.0 kHz were obtained before and at various times after the exposure. Of particular interest were the time course of reflex recovery after the exposure and individual variability with respect to fatigue. A preliminary report has been published (Borg et al. 1979a).

## METHODS

The experiments were performed on 18 subjects aged 18 to 47 years. Their hearing thresholds were within 20 dB of ISO 1964 b

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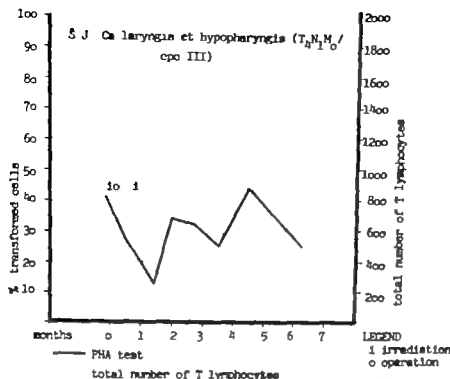


Fig. 7. PHA test and total number of T lymphocytes in a patient following surgery and irradiation.

firm any difference (Fig. 3). In another 18 patients we determined these values before and after treatment with irradiation and found that PHA percentages and the T lymphocyte number fell to almost half of their previous values (Fig. 4). The difference was statistically significant ( $P < 0.01$ ). Fifteen patients (27 in total) received chemotherapy: 14 after surgery and irradiation and 1 treated with chemotherapy only. The percentages of the PHA test and the T-cell count were also compared before and after therapy and a statistically significant difference was found ( $P < 0.01$ ) (Fig. 5). In two patients we followed the changes in the immunological status during combined therapy; reduced the organism's immune reaction (Figs. 6–7).

### CONCLUSION

Although the immunological surveillance may be a factor in the development of a tumor, we still have to consider it as just one among the variables interacting in the process of tumor destruction (Jurn). As clinicians we believe it absolutely necessary today to perform immune reaction tests in patients with laryngeal

cancer. If these tests are positive, that is within the control range, the prognosis is better. This is particularly important in the local immune response to the tumor. If the DNCB test proves to be negative, this should not be considered a contraindication for surgery; instead other immunological tests should be performed, such as PPD, PHA, and the absolute number of T lymphocytes. As the blood analysis is often necessary for following the course of the disease before and after therapy, so is the control of the immunological status important for the evaluation of the organism's overall condition before or during the administration of various kinds of treatment, such as irradiation and chemotherapy.

### RÉSUMÉ

On suppose que les tumeurs malignes apparaissent plus souvent chez les sujets dont l'immunité cellulaire est insuffisante. L'épreuve cutanée DNCB était positive 40% du nombre total du cancer laryngien au début tandis que chez les sujets normaux la positivité de cette réaction cutanée est présente dans 85%–95% des cas. Les lymphocytes T ainsi que leur produits – lymphokines et les facteurs de transfert – jouent un rôle essentiel. Bien que les B – lymphocytes avec leurs anticorps humoraux entraînent un développement tumoral plus rapide une réaction entre les lymphocytes T et lymphocytes B est

**Procedures** After otoscopic examination and hearing threshold determination, stimulus-response curves were determined for 0.5 kHz and 2.0 kHz pure tones. During the 30 min noise exposure the contralateral response was continuously recorded. The balance of the impedance bridge was continuously controlled and the subject was instructed to swallow repeatedly. After the end of the exposure the stimulus-response curves were again obtained according to the following schedule: Immediately after the end of the noise 2.0 and 0.5 kHz; after 5 min. 2.0 kHz; after 10 min. 2.0 and 0.5 kHz. Finally tympanometry and pure tone audiometry were repeated.

## RESULTS

The first and last minutes of the exposure noise were identical. This allowed the detection of even minor changes in reflex characteristics. Fig. 1 shows a typical example of such a sequence of recordings. The upper graph shows a recording of the noise signal wherein the high intensity periods appear diffuse. The middle graph shows the impedance change in the contralateral ear during the first minute and the lower graph shows the corresponding recording during the last minute after 30 min. It is seen that the impedance change well follows the variations in noise intensity. It is especially notable that the impact noise elicits a series of responses which summate to give a background level of muscle activity with superimposed peaks. The impedance change is altered to some extent after the 30 min exposure. Only the lower level components are depressed, stronger sounds still give maximum responses.

The stimulus-response curves obtained at various times post-exposure were compared with the corresponding curves obtained before exposure in order to quantify the alteration of the reflex and to establish the rate of recovery. Fig. 2 shows typical stimulus-response curves before (continuous line) and less than

one minute post-exposure (broken line). The stimulus-response curve after exposure is more or less parallel although shifted to the right and the maximum is unchanged. A shift of this type is compatible with a selective suppression of the low level activity as shown in Fig. 1.

In order to quantify the changes of the reflex the shift of the stimulus-response curve (B in Fig. 2) was measured and expressed as a function of sensation level ( $A$  = dB re reflex threshold Fig. 2). The suppression of the stapedius reflex after 30 min noise exposure is described in this way in Fig. 3. Fig. 3 A shows shifts at 2.0 kHz less than one min post-exposure and Fig. 3 B shows shifts 0.5 kHz about 2 min after exposure. Each subject is shown by a thin continuous line and the mean value  $\pm$  s.e. is shown by the heavy line. It is seen that the variability is appreciable. Some subjects exhibit an improvement of response others suffer more or less pronounced fatigue. The reflex depression was significant ( $P < 0.05$ ) only at 2.0 kHz (5 and 10 dB re reflex threshold). The average shift is largely independent of the sound level which is indicative of a parallel shift rather than a change of slope of the stimulus-response curve. In conclusion there is a small but significant reflex fatigue following an exposure to the 30 min sequence of industrial noise.

To investigate the remarkable spread of data seen in Fig. 3 five subjects were reexposed to the same noise from a few days to one year later and retested.

Fig. 4 shows pairs of curves for each subject representing alterations of reflex properties based on 2.0 kHz pure tone stimulation less than one min post-exposure. The individual mean values are indicated to the right in the graph. It is seen that one subject has consistently large shifts in one direction while another one consistently showed a potentiation of the reflex (negative values). The remaining subjects show intermediate reactions. Although no quantitative estimation of reproducibility can be made the results presented

## GENERAL DISCUSSION

T. Palva – Kastenbauer – Friedmann – Surján Jr – Albegger – Krajina –  
Zechner – Jongkees – Arnold – Wersäll – Naumann

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## THE NORMAL HUMAN MAXILLARY SINUS MUCOSA

*An Electron Microscopic Study*

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**Abstract** The fine structure of the maxillary antral mucosa was studied in 5 cases. The findings revealed the similarity of the nasal and paranasal sinus mucosae. Certain differences were recorded denoting relative diminished activity of the seromucinous glands, diminished exchange between tissue fluid and blood, diminished number of cilia, delicacy and loosening of the epithelium. Thus in inflammation there is lower resistance of the sinus mucosa to infection and easier penetration for microorganisms requiring early systemic administration of antibiotics with postural drainage and antral irrigation subsequently in treatment.

The maxillary sinus originates as an outpouching of the nasal mucosa of the infundibulum in the embryo of about 32 mm CRL i.e. 9th week of intra-uterine life (Keith 1949). It expands progressively occupying more of the future maxillary body. The lining epithelium is derived from the olfactory epithelium of the middle nasal meatus and therefore undergoes the same pattern of differentiation as does the respiratory segment of the nasal epithelium proper (Bhaskar 1976).

Light microscope studies have established the similarity of the nasal and paranasal sinus mucosae (Bradbury 1973, Leonhardt 1977) though the following differences have been reported: the mucosa is thin and less specialized, the epithelium is lower and contains fewer goblet cells, a basement membrane is for the most part not visible, the glands are fewer and smaller, and the venous erectile plexus is absent (Arey 1971).

The ultrastructure of the normal human nasal mucosa has been described by various authors: the epithelium by Tremble (1955, 1962, 1967), Rhodin (1957, 1959, 1963),

Spoendlin (1959), Ricci & Gasparini (1960, 1964), Burian & Stockinger (1963), the vascular bed by Caura & Hinderer (1969), Caura (1970), the seromucinous glands by Toppozada & Gaafar (1973). Of the normal human maxillary sinus mucosa, only the ultrastructure of the secretory cells of the submucosal glands has been investigated (Vidié & Tandler 1976).

The aim of the present work was to complement the ultramicroscopic study of the human normal antral mucosa and to record any differences from that of the nasal mucosa.

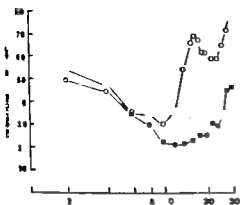
## MATERIAL AND METHOD

Five patients (3 males and 2 females) aged 20-35 years were chosen. They were undergoing dacryo-cystantrostomy surgery but had normal healthy maxillary sinuses. The samples were taken under local surface anaesthesia without adrenalization from the sinus mucosa of all walls. The tissues were fixed in 4% glutaraldehyde solution followed by 1% osmium tetroxide solution buffered at pH 7.4 with phosphate buffer. The tissue blocks were embedded in an Araldite mixture (Luft 1961) and cut with the automated ultramicrotome 111 type 8801A (LKB). The sections were stained with 5% uranyl acetate in 1% acetic acid followed by 0.04% lead citrate. Electron micrographs were prepared in a Siemens Elmiskop I.

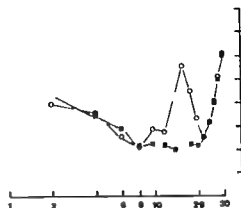
## RESULTS

The ultrastructure of the maxillary sinus mucosa revealed a similarity of the fine details to

GP 63



GP 71



frequency kHz

Fig. 2. Types of hearing loss (■ pre-lesion, ○ post-lesion) induced when the organ of Corti is mechanically disrupted. The type of loss shown by GP71 is termed

notch, while GP65 shows in addition a loss in N sensitivity at the higher frequencies.

Puncturing of the basilar membrane and in some cases Reissner's membrane was carried out in 13 animals. Twelve of these (one was not completely measured on this occasion GP79) showed an immediate loss of sensitivity in the form of a high frequency notch. The maximal loss of the notch immediately post lesion (within 5 min) occurred at frequencies ranging from 13 to 20 kHz with a mean of 16.3 kHz (S.D. 2.0). The magnitude of the loss ranged from 9 to 56 dB with a mean of 28.5 dB (S.D. 15.6).

No significant changes from pre-lesion thresholds up to 8 kHz were evident in the post-lesion electrocochleogram for all animals. In the eight ears where no loss occurred on opening the scala tympani the mean loss after puncturing was 1.6 dB (S.D. 13.1 range 9-46 dB) and occurred at 13-20 kHz (mean 16.6 kHz, S.D. 2.5).

In four ears showing a loss on opening scala tympani the additional effect of puncturing the basilar membrane was studied. Mean loss before puncture was 35.0 dB (S.D. 10.4) at 16.3 kHz (S.D. 0.5) and after puncture 4.1 dB (S.D. 10.7) at the same frequencies. Thus the additional effect of puncturing the basilar

membrane on the hearing loss was 7.1 dB (S.D. 2.7).

#### Post recovery Electrocochleograms

Recovery period varied from 4 to 43 days. Post-recovery electrocochleograms in animals showing initial hearing loss showed improvement in post-lesion threshold in all but 2 of the animals. Three of the animals had a recovery period of 4-8 days, 8 had recovery periods of 17-21 days and 9 had recovery periods of 27-43 days. No significant differences occurred in the amount of recovery between these three groups.

The 4 animals which showed a loss on opening the scala tympani and in which the basilar membrane was not punctured showed a very small recovery (mean 2.5 dB, S.D. 5.3 dB). The eight ears which were punctured and did not have a loss on opening scala tympani showed slightly more pronounced recovery (mean 7.9 dB, S.D. 12.0 dB). Three of the animals included in this group showed complete recovery (Fig. 4). The 4 animals which showed loss on opening scala tympani—and in addition the basilar membrane was punctured—

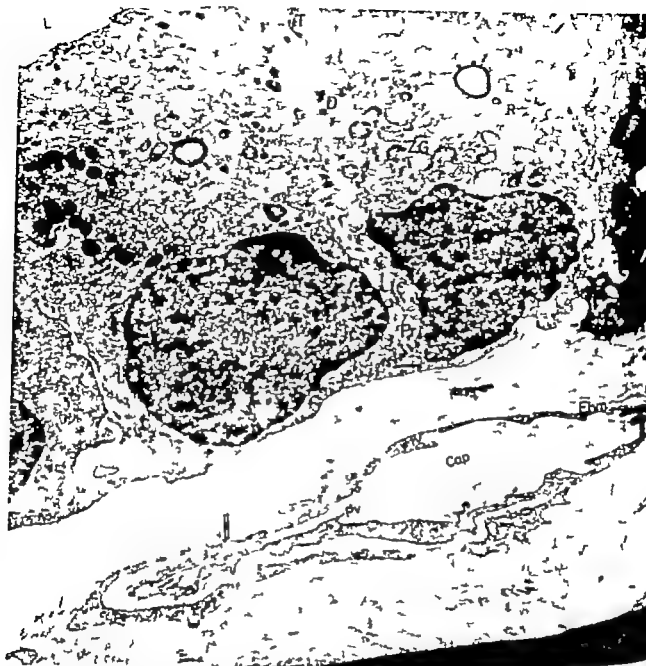


Fig 2 Electron micrograph of a serous gland showing part of the lumen (L) intercellular canaliculi (IC) dilated near the bases of the cells with finger-like cytoplasmic processes (P) many desmosomes (D) and terminal bars (T) The cells contain few zymogen granules (ZG) fewer free ribosomes (R) granular endoplasmic reticulum

(GER) small mitochondria (M) Golgi apparatus (G) and nuclei (N) A periglandular capillary (Cap) is seen with few micropinocytotic vesicles (P) endothelial basement membrane (Emb) with pores (x) and pericyte (Pr)  $\times 9625$

mucous secretory cells had fewer microvilli on their luminal surfaces. The intercellular canaliculi were dilated near the bases of the cells with finger like cytoplasmic processes extending in between them. The canaliculi tapered off and became narrow as they approached the lumen showing more junctional

complexes in the form of desmosomes and terminal bars.

In serous cells (Fig 2) the zymogen granules were fewer in number and paler in density though variations in their densities and sizes existed. A few rounded or oval empty spaces having a definite limiting membrane were seen

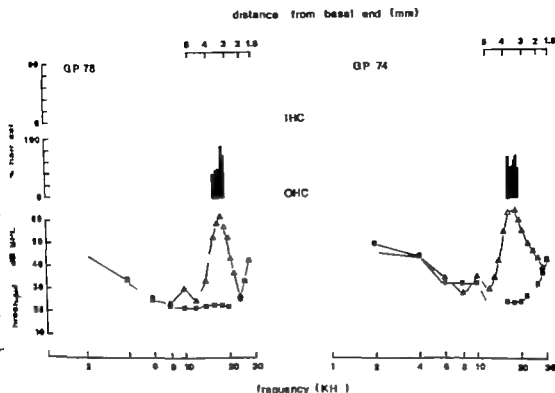


Fig. 5. Upper portion of figure shows hair cell loss (both OHC and IHC) plotted as function of distance from basal end of basilar membrane. Hair cell counts are expressed as percentage loss per 100  $\mu$ m (or groups of 10 inner hair cells). Lower portion of figure shows the N thresholds.

cochleogram ( $\Delta$ , post-recovery  $\square$  thresholds). The relationship between the N thresholds and spatial location of the lesion is explained in the text. GP 74 and 78 both lost only outer hair cells and had no hearing loss on opening the scala tympani: the organ of Corti was not pierced.

venience in estimating the size of the lesion abnormal hair cells were scored as absent. Neither of the 2 sham-operated guinea pigs showed hair cell losses or structural abnormalities in the region of the cochlea in question.

The width of these lesions varied from 70  $\mu$ m to 1.1 mm with an average width of 486  $\mu$ m. In all but 4 animals both inner and outer hair cells were lost or showed severe abnormalities, but in every case more outer hair cells (OHC) were lost than inner hair cells (IHC). OHC loss generally extended both basally and apically beyond the region of IHC loss.

In the 4 animals in which a loss of N sensitivity occurred on opening the scala tympani, hair cell damage was restricted to the outer

hair cells. The loss of OHCs was greatest in the third row and decreased dramatically towards the first (innermost) row. All inner hair cells were present and appeared structurally normal in these animals. An example of SEM results from a lesioned cochlea is shown in Fig. 5. A complete description of the morphology of these lesions is included in a paper in preparation.

#### *Relation between Post recovery Electrocochleograms and Cochlear Morphology*

Examples of the type of correlation found between post-recovery electrocochleograms and hair cell loss are shown in Figs. 6 and 7. In these figures the anatomical lesion has been plotted as a function of distance from the basal





Fig 4 Electron micrograph of a mucous gland showing lumen (L) lined with few microvilli (Mv) intercellular canaliculi (IC) finger-like cytoplasmic processes (P), many desmosomes (D) and terminal bars (T). The cells

contain large pale mucigen granules (MG), fewer free ribosomes (R), granular endoplasmic reticulum (GER), small mitochondria (M), Golgi apparatus (G) and nuclei (N).  $\times 5775$ .

packed droplets showing diminished activity with fewer free ribosomes and smaller mitochondria and Golgi apparatus.

### III The blood vessels

A The capillaries (Fig. 5) There were fewer endothelial cytoplasmic microvilli extending into the lumen. The endothelium contained fewer and smaller micropinocytotic vesicles.

The majority were non-fenestrated except for the periglandular capillaries (Fig. 7). The endothelial basement membrane was frequently porous and in some areas consisted of several incomplete layers. This was more evident in the periglandular capillaries. There were fewer collagenic fibres surrounding the basement membrane.

B The venules (Fig. 6) The endothelium

nition electron microscopy (TEM) may reveal ultrastructural abnormalities within the actual cell body. No studies of this nature were carried out in this experiment.

## DISCUSSION

The sequence of events following mechanical disruption of the organ of Corti and subsequent degeneration of the receptor cells is as yet obscure. Introduction of a 20  $\mu\text{m}$  glass probe through the cochlea partition in this study produced loss of hair cells for distances of up to 11 mm. One possible mechanism which may result in hair cell degeneration is a mixing of the cochlear fluids, perilymph and endolymph. Disruption of the integrity of both Reissner's membrane and the cochlea partition in other studies has produced loss of receptor cells similar to that found in this series of animals (Lawrence 1966; Duvall & Rhodes 1967; Duvall et al. 1969). In our experiments  $N_1$  thresholds were not followed for more than 10 mm after piercing the organ of Corti and consequently it was not possible to assess the final extent of the hearing loss until post-recovery electrocochleograms were measured. Because the final lesion was in all cases much more extensive than the size of the probe, pure mechanical disruption of the organ of Corti seems a remote cause of the final loss of the receptor cells. In any case this method has enabled spatially restricted, reproducible loss of hair cells to be correlated with the resultant deficiencies in the  $N_1$  electrocochleogram.

The use in this study of SEM rather than serial sectioning and surface preparation (Engstrom et al. 1966) allows for a greater degree of accuracy in assessing the morphological condition of the organ of Corti. In serial sectioning, as Bredberg & Hunter-Duvar (1975) have pointed out, every 10th section is generally stained and inspected which means that small lesions may possibly be missed. No such inaccuracy exists in our data. Lesions as small as 3  $\mu\text{m}$  (Fig. 5a) incorporating only 8 OHCs

are clearly visible and the absence of only one hair cell is easily detectable. In addition the high resolution of SEM will reveal surface structural abnormalities which may go undetected with light microscopy. We have assumed that obvious abnormalities in cuticular plates and stereocilia imply abnormal functioning of the hair cell.

Several important points emerge from our results. Firstly the excellent correlation between location of maximum hair cell loss and frequency of maximum loss of  $N_1$  threshold sensitivity provides an additional validation of the place-frequency map of the guinea pig cochlea, even though our lesions were confined by necessity to a fairly restricted region of the basal cochlear spiral. The most complete place frequency maps have hitherto been derived from basilar membrane mechanical measurements (Wilson & Johnstone 1975) and from spiral ganglion recordings (Robertson & Manley 1974; Johnstone 1977) both methods showing substantial agreement. From data provided by Wilson and Johnstone (1975) the equation

$$f = 45e^{-0.271x} \quad (1)$$

can be derived where  $f$  is the cut-off frequency for the basilar membrane tuning curve and  $x$  is distance from the basal end of the basilar membrane. Excellent agreement was found in our present data between the frequency of maximum loss in the  $N_1$  electrocochleogram and the location of hair cell loss when these were aligned according to the above equation. Thus eq. (1) appears to reflect accurately the spatial representation of frequency in auditory nerve fibres. The present  $N_1$  data, together with recent spiral ganglion cell recordings (Robertson et al. in preparation) from our laboratory suggest that the primary nerve fibres at a given location respond best to the frequency corresponding to the high frequency cut-off rather than the peak frequency of the basilar membrane tuning curve at that location (Wilson & Johnstone 1975). This may have im-



Fig 6 Electron micrograph of a venule showing lumen (L) with red blood corpuscles (RBC), thin endothelial cell (E) containing few perinuclear vesicles (Pr) and

continuous endothelial basement membrane (Ebm) and muscle cells (MC) in the tunica media.  $\times 5775$

## DISCUSSION AND CONCLUSION

Although the electron microscope revealed the great similarity of the ultrastructure of the maxillary sinus and nasal mucosae many differences could be identified which help increase our knowledge about this pneumatic cavity.

There was diminished activity of the seromucinous glands as evidenced by

(a) Their scarcity

(b) The diminished number of secretory granules which appeared pale and absent from the lumen and the diminished activity of the cell organelles

(c) As the uptake of serum constituents through the basal lamina into the glands decreased the intercellular canaliculi were dilated near the bases of the cells towards the

ous authors (Kiang et al 1976 Dallos & Harris, 1978 Robertson & Johnstone 1978) that the outer hair cells may in some as yet obscure way be necessary for the normal functioning of the afferent nerve fibres which innervate the inner hair cells.

## ACKNOWLEDGEMENTS

This work was supported by grants from the Australian Research Grants Council and by the Swedish Medical Research Council, project no. 3542. G. Bredberg was supported by a Rasmus Foundation Visiting Professorship. D. Robertson is Queen Elizabeth II Fellow. A. Cody is a Commonwealth Postgraduate Student.

## ZUSAMMENFASSUNG

Kleine getrennte Verletzungen wurden am Cortischen Organ der Meerschneckenohr Cochleae erzeugt, indem feine Sonden getrennt wurden, um eine direkte mechanische Verletzung hervorzubringen. Der elektrophysiologische Zustand der Cochleae wurde durch N-Elektrocochleographie eingeschätzt und der Verlust an Receptorzellen durch Abtastung mit Elektronenmikroskop bestimmt.

Die Hauptbefunde waren: 1) Vertiefliche Übererregung zwischen dem Folcus von Haarzellenverbindungen und der Frequenz- von Hochstempfindlichkeitserhalt an N-Audiotapes. 2) Die mechanische Ausdehnung der mechanisch betroffenen Verletzung scheint wichtiger zu sein als die Gewebezähl der verlorenen Haarzellen für die Bestimmung der Höhe des Verlustes an N-Empfindlichkeit. 3) Haarzellenverluste, die sich über nur 70 µm ausdehnten, konnten als bedeutsame Veränderungen der N-Empfindlichkeit festgestellt werden. Diese Resultate betonen weiterhin die Genauigkeit und Brauchbarkeit des N-Elektrocochleogrammes für die Einschätzung der Leistungsfähigkeit der Cochleae. 4) Verletzungen, die sich nur auf Verletzungen der äußeren Haarzellen betrafen, bewirkten auch starkte Erhöhungen der N-Schwelle.

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Fig. 4. Wedge-shaped replacement of the perist by sclerotic portion advancing by fingers.  $\times 82$ .

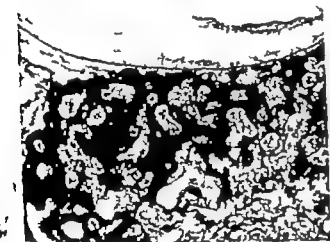


Fig. 5. Replacement of endost, in rosette formation, by spongiotic foci.  $\times 82$ .



Fig. 6. Replacement of endost by foci rich in fibrous component.  $\times 52$ .

sue fluid and the blood as evidenced by the reduced number and size of the endothelial cytoplasmic microvilli and micropinocytotic vesicles the absence of gapping of the interendothelial cells and preponderance of the non fenestrated capillaries. Fenestrae occur where there must be a particularly active and unimpeded exchange between tissue fluid and the blood. Hence in maxillary sinus inflammation early systemic administration of antibiotics is advisable as its diffusion towards the epithelium will help resolution. Antibiotic concentration in the tunica will be less effective should the micro-organism invade deeper layers and may necessitate higher doses.

By way of ciliary beating the mucous blanket lining the epithelial surface moves generally from the sinus interior in the direction of the ostium towards the nasal cavity. In sinusitis clumps of exudate are occasionally too thick and heavy to be readily compressed through the ostium and obstruct the opening or drop back down to the floor (Van Alyea 1970). The diminished number of cilia in the self-cleansing mechanism helps this cycle. The shape of the maxillary sinus as a four-sided pyramid with a quadrilateral base which is facing medially toward the nasal cavity with an ostium at the level of the middle nasal meatus and an apex pointing laterally toward the body of the zygomatic bone adds to the difficulty of the self-cleansing process. Hence the importance of a postural drainage such as the Proetz position and the side position of Parkinson early in the treatment and antrum irrigation later on to remove a large number of micro-organisms from contact with the epithelium having impaired resistance.

The absence of cavernous venous tissue excludes the maxillary sinus as playing any important part in warming of the inhaled air but the mucous blanket passing to the nose helps in the process of humidification.

## RÉSUMÉ

L'ultrastructure de la muqueuse normale du sinus maxillaire fut étudiée dans cinq cas. Les données montrent

la similitude des muqueuses nasale et celles du sinus nasal. Certaines différences furent notées notamment la diminution relative de l'activité des glandes seromucosées, une diminution du nombre des cils, une diminution des échanges entre le fluide ambiant et le sang, ainsi que l'aspect frêle de l'épithélium et son relâchement. En cas d'inflammation la diminution de la résistance de la muqueuse du sinus contre l'infection aide à une pénétration facile des microbes nécessitant l'administration précoce d'antibiotiques avec un drainage postural et irrigation au cours du traitement. L'irrigation de l'acte nasal aide.

## ZUSAMMENFASSUNG

Die feine Struktur der normalen Kieferhöhlenmucosa wurde an fünf Patienten untersucht. Die Ergebnisse zeigten eine große Ähnlichkeit zwischen nasalen und paranasalen Mucosae. Bestimmte Unterschiede zeigen auf relativ abnehmende Aktivität der seromukinösen Drüsen, abnehmenden Austausch zwischen Gewebsflüssigkeit und Blut, abnehmende Zahl der Cilien, Verflüssigung und Erweichen des Epithelums. Sonst geschieht bei Infektionen eine geringere Resistenz des Sinus mucosa gegenüber Infektion und leichtere Penetration für Mikroorganismen, das heißt es wird eine zeitige systemische Verabreichung von Antibiotika mit posturaler Drainage und antraler Irrigation nach der Behandlung erforderlich.

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# THE DIAGNOSIS OF NEGATIVE MIDDLE EAR PRESSURE IN CHILDREN

*The Accuracy of Symptoms and Signs Assessed by Tympanometry*

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**Abstract** Over a period of one year we studied 357 children at random in order to determine whether or not diagnosis of negative middle ear pressure can be made reliably without using tympanometry. Each child was tested repeatedly by screening audiometry at 20 dB and by tympanometry and was examined once by otoscopy. Information about upper respiratory tract infections, feelings of oppression in the ears, and the parents' opinion of the child's hearing ability were obtained from questionnaires. Otoscopy correlated poorly with the tympanometry. Like screening audiometry and the parents' opinion of the child's hearing ability, are more reliable measures. It is concluded that tympanometry is an absolutely necessary tool in the clinical diagnosis of negative middle ear pressure.

Negative middle ear pressure which is the result of an unpaired function of the Eustachian tube occurs very frequently in children (Renvall et al 1978; Fiellau-Nielsen et al 1977). If it is persistent secretory otitis media (SOM) may develop a condition which is characterized by a nonpurulent middle ear effusion. Because the results of today's treatment of SOM are not satisfactory as stated by Tos & Poulsen (1976) early diagnosis and treatment of this problem if possible before the effusion has developed would be desirable.

Impedance audiometry or tympanometry has gained widespread use in otology as a means of documenting the middle ear pressure objectively. The frequency with which this technique is used seems to vary considerably among otologists, and its use is very limited among colleagues in other specialties such as pediatricians and family doctors. Thus some

doctors may claim that the clinical diagnosis of middle ear pathology (i.e. of negative pressure) can be made with sufficient reliability without tympanometry.

In order to evaluate the truth of this assumption it is necessary to examine what is meant by a diagnosis. For this purpose the elements of the clinical diagnosis may be defined as symptoms and signs. Symptoms are those concomitants of the disease or clues to the disease about which the patient can give positive statements while signs may be observed or measured by the physician. Thus the clinical diagnosis may be analysed by evaluating each symptom and sign separately but without analysing the complex interactions of all elements together.

This principle was applied to an epidemiological study conducted during 1977 of negative middle ear pressure in a randomly selected group of children. The signs of middle ear disease were evaluated from tympanometric audiometric and otoscopic observations and some corresponding symptoms of middle ear pathology were obtained from questionnaires answered by the parents.

## METHODS

The study involved children from six schools in Vejle which is an industrial town of about 50 000 inhabitants. From 16 classes a total of 357 children were selected, all of whom were



## IgA TRANSPORT MECHANISM THROUGH THE HUMAN NASAL MUCOSA AN IMMUNOENZYMATIC ULTRASTRUCTURAL STUDY

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**Abstract** The role of secretory IgA antibodies in the antiviral and antibacterial defense of the higher respiratory tract has been widely demonstrated and the IgA determination in the nasal secretion has become of the highest value in the clinical study. The mechanism by which IgA reaches nasal secretion through the mucosa is still a matter of discussion and neither hypothesis put forward by Tomasi (i.e. free filtration through the epithelial-connectival interstitium or transcellular transport plus active secretion) has so far been fully demonstrated. By using a horseradish peroxidase labeled IgG directed against human IgA we have performed with suitable controls a semi-quantitative study on semi-thin and thin section of the IgA behaviour at the level of human nasal mucosa. We have shown that IgA made up and secreted in the connectival interstitium by specific plasma cells, would be taken up by endocytosis by epithelial cells where it can be seen especially in the Golgi apparatus and in the large secretion granules. Since in the intracellular transport of pinocytotic vesicles actin microfilaments and microtubules undoubtedly play a major role with ATP utilisation further studies using either physical factors (e.g. exposure to cold) effecting ATP utilisation or chemical ones (e.g. cytochalasin B and colchicine) depolymerizing respectively microfilaments and microtubules should be performed in order to bring further proofs in favor of the active transport IgA mechanism suggested by our present investigations.

Immunoglobulins A constitute the major share (80%) of immunoglobulins present in the secretions on the lining epithelium of the mucous membranes and therefore they are regarded as the first immunological barrier specific against viruses, bacteria and other antigens (Tomasi et al. 1972).

Their total or partial deficiency is associated with various chronic (or acute recurrent) infectious diseases (South et al. 1965). It is long since known that IgA are synthesized in monomeric form (7S) by plasma cells in the lymphatic tissues and above all by those in

the subepithelial connective tissue of mucous membranes. The monomeric form (7S) represents 85-95% of serum IgA. These have a tendency to polymerize as dimers (10S) to trimers (13S), tetramers (15S) and pentamers (17-18S). Plasma cells also produce the J component, a protein of 23 000 MW present in polymers; polymers represent only a small share of serum IgA whereas they are almost all secretory IgA. In detail 80-90% are dimers, 15-10% are polymers (Tomasi et al. 1972).

It has also been shown that secretory IgA apart from the J component have another specific protein component 55 000-70 000 MW the secretory component that they receive when passing into secretions (Jad) (Heremans 1974, Tomasi et al. 1977).

This different structure of serum IgA as compared to secretory IgA and the preferential concentration of polymers into secretions has made one think about a specific transport mechanism from the blood or from the interstitial space of subepithelial connective tissue into the various secretions through the epithelium.

Tomasi et al. (1968) have proposed two mechanisms from the very beginning: the first one includes the passage of IgA through cellular junctions as far as the surface of the epithelium where they are found in the secretions. This mechanism however would not explain the difference between the dimeric form in the serum and that in the secretions and therefore the presence of the secretory component. Furthermore it was later (Pap-

have analysed the prognostic abilities of more specialized methods of otoscopy such as the use of the operating microscope (Axelsson & Lewis 1976) which showed a very high accuracy rate. Roeser et al (1977) compared the assessments of an experienced otologist and a pediatrician each of whom examined the children with a pneumatic otoscope and impedance measurements. They found poor agreement between the results of otoscopy and impedance measures as well as poor inter-examiner agreement.

The best relative liability was found concerning the parent's assessment of the child's hearing ability and concerning the results of screening audiometry however the sensitivity of these assessments was only about 25%. The reports in the literature on screening audiometry are difficult to compare as the criteria for abnormality vary. In general the results from our study seem to be in agreement with those of most other investigations (Boery et al 1975, Brooks, 1973).

Only about one-fourth of the measured pathological middle ear pressures were accompanied by positive symptoms and signs as appears from Fig. 1 as the 3.7% of the measurements with both features vis-à-vis the total of 13.4% of the measurements that showed pathological middle ear pressures. So this simplified analysis shows that the symptoms and signs which we evaluated are not good prognosticators of negative middle ear pressure below  $-160 \text{ mmHg}$  besides being a possible precursor of SOH. A pressure in that range should be considered a possible hearing handicap (Lidholdt et al 1979). Accordingly the recognition of a negative middle ear pressure is important. This study shows the limited value of simple otoscopy in this diagnosis. This is especially significant because of the importance attributed to this procedure by otologists. In fact, more reliable information may be obtained by asking parents about their child's hearing ability or by recording a screening audiogram than may be obtained by otoscopy.

This study confirms the conclusion of many other investigators that impedance audiometry is an absolutely necessary tool in the clinical diagnosis of negative middle ear pressure.

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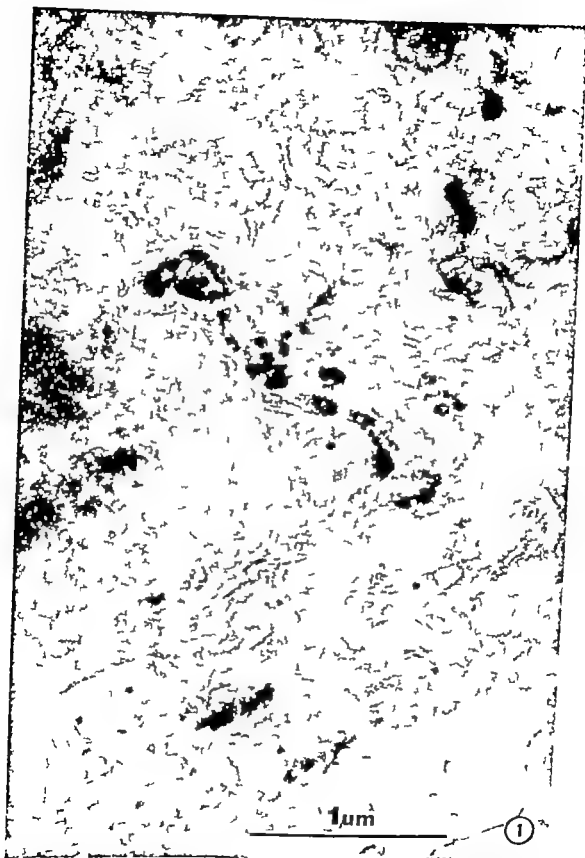
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## ZUSAMMENFASSUNG

In einer Periode von einem Jahr haben wir 357 zufällig ausgewählte Kinder untersucht, um herauszufinden, ob es möglich ist, eine zuverlässige Diagnose eines negativen Mittelohrdrucks zu stellen ohne Tympanometrie zu verwenden. Jedes Kind wurde wiederholt durch Screening Audiometrie bei 20 dB sowie bei Tympanometrie untersucht und wurde einmal durch Otoskopie untersucht. Anzeichen über Infektionen in dem oberen Respirationstrakt, das Gefühl eines Drucks in den Ohren und die Beurteilung der Eltern über das Gehörvermögen des Kindes wurden durch Fragebogen ermittelt. Otoskopie bildete eine schlechte Korrelation mit Tympanometrie während Screening Audiometrie und die Beurteilung der Eltern über das Gehörvermögen des Kindes ein zuverlässiges Maß anzeigte. Daraus folgert, daß Tympanometrie ein absolut notwendiges Gerät der klinischen Diagnose eines negativen Mittelohrdrucks ist.

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*Fig. 1* Human nasal mucosa treated with IgG anti-human IgA+HRP complex. The reaction product (DAB) can be seen in great quantity throughout the Golgi apparatus, both on the cisternae of dictyosomes, and in the vesicles. Unstained section  $\times 45\,000$ .



Fig. 3 Scintigram of patient II presented according to Fig. 1. Increased accumulation in the left mastoid region.

strated an abnormally high accumulation of the isotope corresponding to the right mastoid region and upper lateral part of the right external meatus which areas were left intact at earlier surgery (Fig. 2). The patient was then irradiated with 60 Gy in fractionated doses. A second scintigraphy 2 weeks after the last irradiation dose showed an increase of the pathologic isotope accumulation. The patient died from recurrent carcinoma of the ear and bronchopneumonia in March 1977.

#### Patient II

A man aged 75 without history symptoms or signs of chronic otitis. In 1961 a basal cell carcinoma of the skin developed behind the left auricle. The tumour was excised, but from 1966 to 1976 local recurrences appeared and were excised five times. In 1972 the peristoma of the mastoid process was excised in connection with the skin excision but the bone was left intact. In July 1976 a 4×4 cm tumour was palpated behind the ear under the skin and against the bone. Histology demonstrated a recurrence and the histopathological diagnosis was changed to squamous cell carcinoma. Radiography did not demonstrate any destruction but tomography demonstrated a destruction of bone under the tumour probably not explained by the excision of peristoma in 1972.

Scintigraphy was performed on the same day as the tomography and revealed an abnormally high radionuclide accumulation in the

left mastoid region corresponding to the tumour recurrence and the destruction demonstrated by tomography (Fig. 3). The patient was irradiated with 40 Gy in fractionated doses. A second scintigraphy 2 weeks after the last irradiation dose demonstrated an increase in the pathologic isotope accumulation. The patient was operated by radical mastoidectomy but in February 1979 again a recurrence was verified by histology.

#### Patient III

A woman aged 72 with symptoms of chronic inflammation of the left middle ear since childhood with periods of excretion from the ear. A chronic central perforation had been present at least for the last 5 years, but the ear had been dry during this period. Since December 1975 excretion and slight pain had been present in the ear. At examination the internal parts of the external meatus were filled with abnormal soft tissues possibly engaging the middle ear. No symptoms of cochlear or vestibular engagement were present. Histologic examination of small biopsies revealed a highly differentiated squamous cell carcinoma. Ordinary radiography demonstrated a dense cell system and absence of bone details in the floor of the external meatus.

Tomography of the ear also demonstrated the bone destruction of the floor of the external meatus. The hearing bones could not be identified and the air space of the middle ear

Table I *Semiquantitative evaluation of osmium black reaction product in human nasal m*

|   | Anti-IgA<br>antibody<br>+HRP<br>(%) | Anti-IgG<br>antibody<br>+HRP<br>(%) | DAB only<br>(endogenous<br>peroxidase)<br>(%) |
|---|-------------------------------------|-------------------------------------|---|
| % of cells with reaction product              | 100                                 | 30                                  | 28  |
| Golgi apparatus, w/t reaction product         | 100                                 | 30                                  | 28  |
| Secretory large granules w/t reaction product | 80                                  | 3                                   | 3   |

somes were completely free of electron-dense osmium black products. The same reaction product was neither visible in the smooth endoplasmic reticulum nor in the rough endoplasmic reticulum mitochondria or in the nucleus. This could account for a scarce role of this organelle in direct transport mechanism of the secretory IgA.

Both control specimens presented a few cells with reaction product (Table I) but it was localized in the Golgi apparatus or in its small vesicles in a very low quantity in comparison with the specimen treated with the complex containing the anti IgA antibody.

The results we have attained show that IgA are preferentially located in quantities that can be evidenced with immunoenzymatic techniques in the Golgi apparatus and in the big secretory granules localized in the apical zone of epithelial cells of the nasal mucosa.

Therefore it is likely that these two organelles are involved in the transport mechanism of IgA. However it is not possible to exclude that even other organelles directly or indirectly intervene in this mechanism for the fact only that they do not have the electron-dense products of the peroxidasic reaction; this could in fact be explained by the fact that IgA are scarcely concentrated here or that their turn-over (passage) is very rapid.

On the other hand it has already been shown that RER or polyosomes are responsible for the synthesis of the secretory component and it is also known that proteins synthesized by RER are started off to secretion through the Golgi apparatus (Russo 1977).

Therefore the diagram shown in Fig. 1 represents a possible sequence of events in the transport mechanism through the epithelial cells of the nasal mucosa, based on the data obtained by one of us and other authors. It is analogous to the mechanism of polarized transport and active transport (Russo 1977; Vassilatos & Russo 1979).

IgA molecules would be engulfed by endocytosis from the interstitium at the apical pole of the epithelial cell in the lamina propria. The pinocytotic vesicles migrate toward the Golgi apparatus, the vector of their movement which indicates the cell polarity is determined by the architecture of membrane associated microfilaments (Clarke & Spudis 1977). In the Golgi region the pinocytotic vesicles meet the secretory component coming from the RER. The functional tendency of the Golgi apparatus to concentrate molecules to form secretory granules made the IgA here immunologically demonstrable. Indeed at Golgi apparatus IgA join to other secretory products in the formation of the large secretory granules.

As an obscure point remains the reason why IgA can be shown in the large granules with a medium-density homogeneous content, whereas they have not been seen in the small granules having double density (Fig. 1). It is probable that the latter have not the ability to fuse their membranes with the vesicles coming from pinocytosis and do not come in contact with the secretory component.

The mechanism we have proposed is analogous to that shown for other epithelial cells and in particular in the hepatic cell

# Case VI

A woman aged 40 suffering from external otitis of the left ear since February 1979. In July biopsy was taken demonstrating a moderately differentiated cancer of the ear and the patient was referred for surgery. Radiography August 1979 demonstrated a slightly increased attenuation in the lateral part of the mastoid cell system of the affected ear. Polytomography revealed a destruction of the bony part of the external meatus as well as of the temporomandibular joint fossa and the anterior part of the mastoid process.

Scintigraphy demonstrated a definitely increased accumulation of  $^{99m}\text{Tc}$ -DP in corresponding regions, but the area affected according to the scintigraphic results was larger than according to the radiographic results (Fig. 8).

The patient was operated one week later and the tumour was found to be growing extensively in the temporal bone and into the zygomatic region.

## DISCUSSION

The patients (I, II, III, V and VI) with verified squamous cell carcinoma of the ear and bone destruction demonstrated by tomography had abnormally high accumulation of  $^{99m}\text{Tc}$ -DP in the temporal area. One patient with a basal cell carcinoma (IV) had no demonstrable bone destruction and a normal bone scintigram. The abnormalities were differently shaped in the gamma camera images which indicates a possibility of differentiating between different extensions of neoplastic growth in the temporal bone. However, inflammation as well as neoplastic processes influence on bone accumulation of  $^{99m}\text{Tc}$ -DP and abnormal accumulation patterns could have several explanations, for instance neoplasia, inflammation or both (Greenberg et al., 1968; Galasko & Doyle 1977; Tofe et al. 1975; Treves et al. 1976; Bergstedt & Haverling, 1978; Bergstedt & Haverling, 1978; Bergstedt & Haverling, 1978).

The abnormal accumulation in the mastoid region of patient I (Fig. 2) might depend on inflammatory reactions provoked by earlier surgical excision of the auricle and the cartilaginous part of the external meatus or by the small biopsies taken later from this area. However, biopsy demonstrated a recurrence of the tumour growth and the mastoid region and the bony part of the external meatus had been left intact by the surgeon. It is therefore more likely that the abnormal accumulation was caused by a spread of malignant growth into the cortex of the mastoid process which also explained the destruction demonstrated by tomography.

The abnormal accumulation in the mastoid region of patient II was most probably explained by the tumour recurrence verified by histopathological examination which had changed the original diagnosis from basal cell to squamous cell carcinoma. However, it cannot be excluded even if not probable that the pathologic uptake of isotope could be explained by the excision of peristoma of the region in 1977 and therefore this case also illustrates the necessity of careful analysis of scintigraphic findings in correlation to patient history in order to lessen the risk of false-positive diagnosis of neoplastic engagement of bone tissue.

The engagement of the whole left pyramid of patient III indicates an influence on bone far beyond the radiologically demonstrable bone destruction of the tumour. This patient had a chronic otitis of the left ear and the abnormal accumulation of the isotope might in part be explained by the chronic inflammatory reactions. However, no acute exacerbations had occurred during the last few years and so the abnormal accumulation was presumably explained by neoplastic influence on the bone. Despite the macroscopically radical excision of the tumour area the patient eventually died from local recurrence. The scintigraphy findings probably correlated better to tumour extension than did the tomography findings.

The basal cell cancer tissue of patient IV

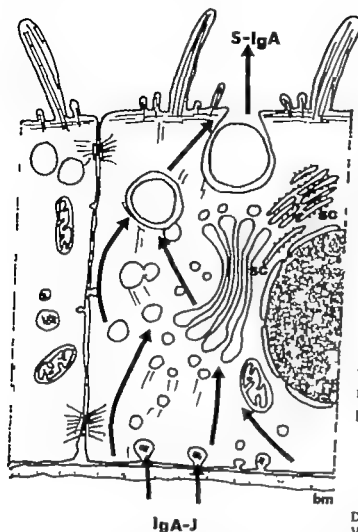


Fig. 4 Diagram showing our hypothesis on IgA transport mechanism through the mucosal epithelial cell. IgA-J, IgA and J component arrive to the Golgi zone via pinocytosis. S-C secretory component is synthesised by endoplasmic reticulum. IgA-J-SC dimer-complex are formed at Golgi level in the large granules. Large granules are released at the cell surface (secretory pole). BM: basal membrane.

other polarized cells where an active and continuous flow has been shown from the interstitial space to the biliary duct (external space) via Golgi apparatus (Russo et al. 1977).

Analogously, this mechanism should be energy-dependent for IgA as well and strictly related to the integrity of the contractile internal system of the microfilaments and microtubules (Russo et al. 1977).

Therefore, it should be inhibited by all those physical or chemical stimuli which inhibit the utilisation of ATP or interfere with the function of microfilaments and microtubules de-

polymerizing them (Russo et al. 1976, Van Rossum & Russo 1979). Further studies are in progress to clarify the latter and other aspects. In particular, we are studying the effects of low temperature on IgA level in the secretions (it is in fact well known that respiratory infections have a higher rate during winter probably as a result of the weakening of the immunological barrier represented by secretory IgA as their mechanism of transport is certainly slowed down or inhibited by cold through a reduction of the utilisation of ATP and of local administration of cytochalasin and colchicine (which depolymerize actin microfilaments and microtubules respectively) (Van Rossum & Russo 1979). The IgA decrease in the secretions under such conditions would be a sufficiently strong argument itself in favour of the mechanism we have proposed.

## ZUSAMMENFASSUNG

Die Rolle der ausgeschiedenen IgA Antikörper in der Verteidigung gegen Viren und Bakterien in den oberen Atemwegen ist schon allgemein demonstriert worden. Wir halten die Dosierung in der Nasensekretion als eine wichtige Probe für klinische rhinologische Untersuchungen. Der Mechanismus, durch den IgA durch die Mukose in die Nasensekretion gelangt, ist unbekannt und beide von Tomasi aufgestellten Hypothesen (freie interstitielle Filtrierung oder transzellulärer Transport und aktive Sekretion) sind bis heute nicht durch experimentelle Versuche unterstützt. Mittels mit horseradish-Peroxidase markierten IgG gerichtet gegen menschliche IgA und den geeigneten Kontrollen, haben wir semiquantitative Studien über das Verhalten der IgA in halbdünne und dünne menschliche Nasenmukose-Schnitten durchgeführt. Dadurch wurde bewiesen, daß IgA synthetisiert und in den konnektiven Zwischenraum von den spezifischen Plasmazellen ausgeschieden durch Endocytose von den epithelialen Zellen, wo sie besonders in Golgi und in den großen Sekretionsgranulen reichert aufgenommen wird. Da in dem intrazellulären Transport der pinocytotischen Vesicula bestimmt auch Actin-Mikrofilamenten und Mikrotubula mit gleichzeitiger ATP-Benutzung entwickelt sind, erschienen als interessante weitere Versuche die durch physikalische (z. B. Kälte) oder chemische Mittel (z. B. Cytochalasin oder Colchicin) respektive die Benützung der ATP hemmend oder Mikrotubulern und Mikrotubula depolymerisierend weitere Proben zugunsten des durch diese Versuche als wahr erscheinenden aktiv Transport Mechanismus der IgA bringen würden.

Table 1 Patients with prolonged bleeding time

| Patient | Age (years) | Ivy bleed ing time (minutes) | Cause of the prolonged bleeding | Treatment                |
|---------|-------------|------------------------------|---------------------------------|--------------------------|
| I C S   | 13          | 19                           | Thrombasthenia                  | AMCA + fresh whole blood |
| E R     | 22          | 18                           | No definite diagnosis           | AMCA                     |
| J J H   | 22          | 15                           | Thrombasthenia                  | AMCA + fresh whole blood |
| O K     | 47          | 14                           | Thrombasthenia                  | AMCA                     |
| S I H   | 32          | 14                           | Enhanced fibrinolytic activity  | AMCA                     |
| K O     | 49          | 18                           | Intake of acetyl-salicylic acid | 0                        |
| R M     | 52          | 14                           | Intake of acetyl-salicylic acid | 0                        |
| P H     | 5           | 16                           | Intake of acetyl-salicylic acid | 0                        |
| B R     | 79          | 18                           | Intake of acetyl-salicylic acid | AMCA                     |
| L M     | 40          | 13                           | No definite diagnosis           | AMCA                     |
| M D     | 41          | 17                           | Unknown                         | 0                        |
| M E     | 62          | 18                           | Intake of acetyl-salicylic acid | AMCA                     |
| A L     | 69          | 16                           | Unknown                         | 0                        |
| I L     | 51          | 14                           | Intake of acetyl-salicylic acid | AMCA                     |
| M M     | 57          | 13                           | Thrombasthenia                  | AMCA                     |
| A V     | 8           | 18                           | No definite diagnosis           | AMCA                     |
| K P     | 33          | 18                           | Intake of acetyl-salicylic acid | 0                        |
| L L     | 8           | 14                           | Unknown                         | 0                        |
| T C     | 15          | 15                           | No definite diagnosis           | AMCA                     |
| G P     | 20          | 14                           | No definite diagnosis           | AMCA                     |
| M M     | 9           | 18                           | Intake of acetyl-salicylic acid | 0                        |
| K N     | 55          | 18                           | No definite diagnosis           | AMCA                     |
| I S     | 38          | 14                           | Intake of acetyl-salicylic acid | AMCA                     |
| J O     | 5           | 18                           | No definite diagnosis           | AMCA                     |
| U L     | 49          | 18                           | Intake of acetyl-salicylic acid | 0                        |

Platelet count was done according to Björkman (1959) and Duke bleeding time as described by Nilsson (1974). Platelet adhesive tests according to Hellm's method (Hellm 1960). Aggregation studies of the platelets with Born's aggregation method were performed in a Payton Dual Channel Aggregation Module as described by Cronberg (1970).

**Coagulation:** Coagulation time in glass tubes and in plastic tubes, recalcification time of plasma and activated partial thromboplastin time (APTT) (General Diagnostics) (Nilsson 1974) were studied. Factor VIII clotting activity (VIII C) was determined biologically as described by Nilsson et al. (1957). Factor VIII related antigen (VIII R Ag) immunochemically in the way described by Holmberg & Nilsson (1973). Factor IX, one stage prothrombin time (P&P), factor V, fibrinogen, factor XIII (fibrin stabilizing factor) were determined by methods reported earlier (Nilsson 1974, Henriksson et al. 1979).

**Fibrinolysis** was tested by determination of fibrinolytic activity of plasma and resuspended *esoglobulin* precipitate on fibrin plates, *esoglobulin* clot lysis time and fibrin degradation products (FDP) (Nilsson 1974, Nilsson & Olow 1962).

The first Ivy test was performed on the day before operation. If the bleeding time was prolonged surgery was usually postponed for 2-3 weeks during which time a re-test and further investigation were performed.

## RESULTS

In 25 patients (8.3%) we found a prolonged bleeding time (Table 1). In 10 cases the condition could be related to intake of acetyl-salicylic acid since the bleeding time at re-testing after withdrawal of the drug was normal. In 3 more cases with no history of acetyl-salicylic acid intake the bleeding on repeated assay also was normalized. In the remaining



## CYTOTOXIC LEUKOCYTE REACTION

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**Abstract** Blood samples from 49 subjects were submitted to cytotoxic leukocyte tests which were read double blind by two laboratory technicians. The results were identical in 78% of the tests and the statistical error of the method was 0.46. In a group of 32 subjects the cytotoxic test was repeated on 3 consecutive days. Deviations in the results occurred in 18-70% of the tests, the statistical errors varying between 0.38 and 0.43. In a series of serum samples (75 subjects) with positive leukocyte reactions to food antigens various in vitro procedures were carried out in connection with the cytotoxic test. The following observations were made. In 4 of 58 tests a positive leukocyte reaction was transferred with the reactive serum to cells from non-reactive subjects. After heating of the reactive serum (56°C) and washing of the cells reactivity was no longer observable in 44 out of 57 tests. Treatment with EDTA consistently blocked expected cytotoxic reactions in 36 tests. DSCG inhibited expected reactions in 45%, antihistamine in 93% and cortisone in 70% of the tests.

The cytotoxic test is based upon the observation that food antigens affect the amoebic movement and streaming of cytoplasmic granules and in stronger reactions the viability of neutrophilic leukocytes. Black (1956) was the first to describe this observation and later the Bryans (1960, 1971) developed a test for clinical use based on this phenomenon. The mechanism underlying the cytotoxic leukocyte reaction has been rather neglected. Only Black's experiments suggest that the reactivity is a property of the patient's serum. Two questions have frequently been raised in the publications on the cytotoxic test: the reliability of test readings and the reproducibility of the test (Lieberman et al. 1975; Benson & Arkins 1976).

## MATERIAL AND METHODS

1 Blood samples for cytotoxic tests were drawn in a group of 49 patients for the purpose of studying the reliability of test readings. The testing technique was exactly the same as that reported by Bryan & Bryan (1960). Cytotoxic tests were carried out with 23 food antigens, a total of 1 127 tests, and the reactions were read in a double-blind manner by two laboratory technicians.

2 Another series of blood samples was collected from 32 patients in an effort to study the reproducibility of the test on consecutive days. The test with 23 food antigens was repeated on 3 successive days and the results recorded blind by the same laboratory technician.

3 In order to analyse factors which may influence the cytotoxic leukocyte reaction, following in vitro procedures were used in connection with cytotoxic tests on blood samples from 75 subjects.

(a) The cells from non-reactive subjects were mixed with serum from persons with positive cytotoxic leukocyte reactions to food antigens. The mixing procedure was repeated in reverse order with reactive cells and non-reactive serum.

(b) Reactive serum was heated in a 56°C water bath for 30 min and 2 h, respectively, to inactivate complement and IgE. In this series of tests, first unwashed cells and then cells washed twice for 15 min with TRIS-BSS buffer

## ATTIC RETRACTIONS FOLLOWING SECRETORY OTITIS

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(Received May 30, 1979)

**Abstract** At re-examination of 577 ears with secretory otitis 1 to 8 years after tubulation otomicrosurgery and tympanometry revealed different types of retraction of Shrapnell's membrane in 34% of these 4.2% had pronounced retraction with retraction of the osseous annulus and 0.2% had atrophic cholesteatoma. There was a close correlation between the frequency and severity of retraction on the one hand and the age of the patient at re-examination, the tympanometric conditions, and the etiology of pars tensa on the other. The continued regression is further promoted by lack of natural healing of the retraction. As the frequency of secretory otitis is very high in children the 4.2% of pronounced retractions constitute a sufficient quantitative basis for their progression to atrophic cholesteatoma.

The principal purpose of this study was to establish the character and frequency of pathological changes in the Shrapnell's membrane in well-treated secretory otitis. Another long-term object has been to investigate whether atrophic cholesteatoma is a direct after-effect of secretory otitis or whether it develops directly from retractions in Shrapnell's membrane.

*Earlier investigations*

Several investigators have studied sequelae changes of pars tensa following secretory otitis (Stevens 1962, McKinnon 1971, Kulby et al 1972, Mawson & Fagan 1972, Kokko 1974, Bonding & Lorentzen, 1973, 1974, Tos & Poulsen, 1976) but to our knowledge there exists no systematic investigation of the changes in Shrapnell's membrane.

Mawson & Fagan (1972) found attic retractions in 2% of ears after well-treated secretory otitis. Kokko (1974) found attic cholesteatoma in 0.7% of ears and McKinnon (1971) in 1.0% when including both attic and sinus cholesteatoma.

In our oldest material comprising patients with secretory otitis treated with tubulation before 1970 and re-examined in 1975 i.e. 5 to 8 years after treatment, we found changes of pars tensa in 55% of ears (Tos & Poulsen 1976) and cholesteatoma in 1%. In addition we found different degrees of retractions of pars tensa. Unfortunately this material was not systematically investigated with reference to changes in Shrapnell's membrane. Consequently in 1977 and 1978 we re-examined a new material of patients operated upon from 1971 to 1975 and after systematic investigations of the changes in Shrapnell's membrane these were divided into predetermined types of retraction.

## MATERIAL AND METHOD

The material comprises 577 ears from children under the age of 13, the majority being between 4 and 8 years of age with secretory otitis treated under general anaesthesia with adenotomy, myringotomy and excision of secretion and most frequently with tubulation.

The children were most often referred from an ENT specialist and our routine method has been to insert an Armstrong tube anteriorly or anterior inferiorly in the drum. In 58 ears tubulation was not performed but only myringotomy. Ten ears had much mucous secretion on this side but were not tubulated in deference to the wish of the parents. 7 ears had some serous secretion and 31 ears had small or negligible amounts of mucous secretion for which reason we estimated that tubulation was unnecessary. The patients were regularly

## CYTOTOXIC LEUKOCYTE REACTION

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**Abstract** Blood samples from 49 subjects were submitted to cytotoxic leukocyte tests which were read double blind by two laboratory technicians. The results were identical in 78% of the tests and the statistical error of the method was 0.46. In a group of 32 subjects the cytotoxic test was repeated on 3 consecutive days. Deviations in the results occurred in 18-20% of the tests, the statistical errors varying between 0.38 and 0.43. In a series of serum samples (75 subjects) with positive leukocyte reactions to food antigens various in vitro procedures were carried out in connection with the cytotoxic test. The following observations were made: In 42 of 48 tests a positive leukocyte reaction was transferred with the reactive serum to cells from non-reactive subjects. After heating of the reactive serum (56°C, 2 h) and washing of the cells reactivity was no longer observable in 44 out of 5 tests. Treatment with EDTA consistently blocked expected cytotoxic reactions in 36 tests. DSCG inhibited expected reactions in 45%. antihistamine in 93% and cortisone in 70% of the tests.

The cytotoxic test is based upon the observation that food antigens affect the amoebic movement and streaming of cytoplasmic granules and in stronger reactions the viability of neutrophilic leukocytes. Black (1956) was the first to describe this observation and later the Bryans (1960, 1971) developed a test for clinical use based on this phenomenon. The mechanism underlying the cytotoxic leukocyte reaction has been rather neglected. Only Black's experiments suggest that the reactivity is a property of the patient's serum. Two questions have frequently been raised in the publications on the cytotoxic test: the reliability of test readings and the reproducibility of the test (Lieberman et al. 1975, Benson & Arkins 1976).

## MATERIAL AND METHODS

1 Blood samples for cytotoxic tests were drawn in a group of 49 patients for the purpose of studying the reliability of test readings. The testing technique was exactly the same as that reported by Bryan & Bryan (1960). Cytotoxic tests were carried out with 23 food antigens, a total of 1 127 tests, and the reactions were read in a double-blind manner by two laboratory technicians.

2 Another series of blood samples was collected from 32 patients in an effort to study the reproducibility of the test on consecutive days. The test with 23 food antigens was repeated on 3 successive days and the results recorded blind by the same laboratory technician.

3 In order to analyse factors which might influence the cytotoxic leukocyte reaction the following in vitro procedures were used in connection with cytotoxic tests on blood samples from 75 subjects:

(a) The cells from non-reactive subjects were mixed with serum from persons with positive cytotoxic leukocyte reactions to food antigens. The mixing procedure was repeated in reverse order with reactive cells and non-reactive serum.

(b) Reactive serum was heated in a 56°C water bath for 30 min and 2 h, respectively, to inactivate complement and IgE. In this series of tests, first unwashed cells and then cells washed twice for 15 min with TRIS BSS buffer

Table VI Relationship between pathology of pars tensa and types of retraction of Shrapnell's membrane

|          | Types of retraction |                  |                   |                    |                  | Chole-<br>steatoma<br>N=1<br>(%) | Total<br>N=527<br>(%) |
|----------|---------------------|------------------|-------------------|--------------------|------------------|----------------------------------|-----------------------|
|          | 0<br>N=346<br>(%)   | I<br>N=67<br>(%) | II<br>N=91<br>(%) | III<br>N=18<br>(%) | IV<br>N=4<br>(%) |                                  |                       |
| normal   | 57.5                | 26.9             | 26.4              | 5.6                | —                | 100.0                            | 45.2                  |
| abnormal | 42.5                | 79.1             | 73.6              | 94.5               | 100.0            | 100.0                            | 54.8                  |

traction of Shrapnell's membrane. Diffuse atrophy with retractions of pars tensa is characterized by a thin retracted ear drum with a diminished tympanic cavity but without adhesion to the promontory. Valsalva's test made the drum bulge forward prominently with Siegle's speculum. It was hypermobile. Retraction of Shrapnell's membrane occurred in 74% of these ears. In adhesive otitis the drum adhered to the promontory, was partly or entirely immobile and the middle ear was partly or entirely atelectatic. There were retractions of Shrapnell's membrane in all ears. Localized atrophy without retraction occurred either in the upper posterior or upper anterior quadrant or downward in tensa. Retraction of Shrapnell's membrane occurred in almost one-third of these ears. Atrophy localized posteriorly with retraction onto the incudostape-

dial joint and a pexi to the joint was accompanied in 88% of the ears by retraction of Shrapnell's membrane. Diffuse tympanosclerosis or fibrosis of the drum characterized a few ears with a considerably thickened drum, in the remaining ears there was circular or horse-shoe shaped tympanosclerosis in the entire circumference of the pars tensa. Localized tympanosclerosis either posteriorly anteriorly or downwards was in 35% of cases accompanied by retraction of Shrapnell's membrane. A combination of tympanosclerosis in one place and atrophy in another occurred frequently and Shrapnell's membrane was retracted in 66% of these ears.

Table VII shows the clear correlation between the severity of the pathology of pars tensa and the frequency of retractions of Shrapnell's membrane in the following order:

Table VII Relationship between different pathological conditions of pars tensa and types of retraction of Shrapnell's membrane

| Condition of pars tensa        | No. of ears | Types of retraction |          |           |            |           | Chole-<br>steatoma<br>(%) |
|--------------------------------|-------------|---------------------|----------|-----------|------------|-----------|---------------------------|
|                                |             | 0<br>(%)            | I<br>(%) | II<br>(%) | III<br>(%) | IV<br>(%) |                           |
| Diffuse atrophy                | 3           | 69                  | 22       | 9         |            |           |                           |
| Diffuse atrophy and retraction | 31          | 26                  | 19       | 48        | 3          |           |                           |
| Adherent otitis                | 13          |                     | 23       | 38        | 38         |           | 3                         |
| Localized atrophy              | 49          | 69                  | 14       | 16        |            |           |                           |
| Localized atrophy and pexi     | 17          | 1                   | 1        | 47        |            | 0         |                           |
| Diffuse tympanosclerosis       | 29          | 45                  | 38       | 15        | 18         | 12        |                           |
| Localized tympanosclerosis     | 79          | 65                  | 14       | 19        | 3          | 5         |                           |
| Atrophy and tympanosclerosis   | 48          | 44                  | 21       | 21        | 13         | 2         |                           |
| Total pathology                | 289         | 50.9                | 18.3     | 33.2      | 5.9        | 1.4       | 0.3                       |
| Normal pars tensa              | 238         | 83.6                | 5.9      | 10.1      | 0.4        |           |                           |

Table III The effect of drugs on the cytotoxic reaction

| Drug                      | No of subjects | No of positive reactions in original test | Inhibited reactions |                |
|---------------------------|----------------|---|---------------------|----------------|
|                           |                |   | n                   | c <sup>a</sup> |
| DSCG                      | 13             | 6   | 28                  | 45             |
| Antihistamine             |                |   |                     |                |
| Phenergan <sup>a</sup>    | 16             | 44  | 41                  | 93             |
| Tavegil <sup>12</sup>     | 16             | 44  | 41                  | 93             |
| Cortisone                 |                |   |                     |                |
| Betnesol <sup>a</sup>     |                |   |                     |                |
| 1 25 000                  | 1              | 31  | 8                   | 19             |
| Solo-Cortef <sup>13</sup> |                |   |                     |                |
| 1 2 000                   | 1              | 31  | 7                   | 3              |
| 1 100                     | 1 <sup>a</sup> | 31  | 16                  | 5 <sup>a</sup> |

the reverse mixing procedure the cytotoxic reactivity of the leukocytes remained almost undiminished in 40% of the tests despite the addition of non-reactive serum. Positive leukocyte reaction was observed in 46% of tests performed with heated reactive serum (56°C for 2 h) reactive non washed cells and food antigens. Incubation with food antigens produced positive reactions in 21% of tests in which heated serum was added to washed reactive cells (Table II). Similar results were obtained when the tests were carried out with serum heated for 30 min.

EDTA was found to block all expected cytotoxic reactions. Control cells i.e. non reactive cells retained their normal mobility when prepared with EDTA solutions.

After addition of one drop of 5% DSCG-solution instead of sterile distilled water expected positive reactions failed to occur in 28 out of 62 testing instances (45%) (Table III). A 1% DSCG solution did not alter any of the test results. The use of higher concentrations of DSCG 10 and 20% did not cause a change in the figures obtained with 5% solution.

Antihistamine preparations inhibited the expected reaction in 42 out of 44 tests (93%) (Table III). Stabilization of the cell membrane occurred in some tests on samples from known reactors also when antihistamine was added 10 min after incubation with food antigen had started.

Cortisone solution in a concentration

1 25 000 blocked the expected cytotoxic reaction in 19% of the tests. Higher concentrations (1 2 000 and 1 200) inhibited the reaction in 23 and 52% respectively (Table III).

4 Histamine solution at a dilution of 1 1 000 did not affect the normal cell mobility. At a dilution of 1 100 some cell rounding and diminished pseudopod activity were observed. When histamine chloride and histamine acid phosphate solutions were used at dilutions of 1 10 and 1 5 almost all cells were rounded.

## DISCUSSION

The cytotoxic reaction is apparently mainly connected with serum factors. A circumstance arguing in favour of this is that the majority of reactions can be transferred with reactive serum to non reactive cells. In our *in vitro* experiments the cytotoxic reactivity was transferred with the serum in 72% of the tests while in Black's series the corresponding figure was 100%. Our observations differ slightly from the implications of Black's results also in tests performed with heated serum. When the two most likely reacting serum factors complement and IgE were inactivated by heating at 56°C for 30 min and 2 h respectively we still noted positive reactions in 21% of the tests. On the other hand we found that EDTA which blocks  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions which are essential in the classical

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| Pars tensa | Types of retraction |                  |                   |                    |                  | Cholesteatoma<br>N=1<br>(%) | Total<br>N=527<br>(%) |
|------------|---------------------|------------------|-------------------|--------------------|------------------|-----------------------------|-----------------------|
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| Normal     | 57.5                | 26.9             | 26.4              | 5.6                | —                | 100.0                       | 43.2                  |
| Adhesive   | 42.5                | 79.1             | 73.6              | 94.3               | 100.0            | 100.0                       | 54.8                  |

retraction of Shrapnell's membrane. Diffuse atrophy with retractions of pars tensa is characterized by a thin retracted ear drum with a diminished tympanic cavity but without adhesion III the promontory. Valsalva's test made the drum bulge forward prominently with Siegle's speculum it was hypermobile. Retraction of Shrapnell's membrane occurred in 74% of these ears. In adhesive otitis the drum adhered to the promontory was partly or entirely immobile and the middle ear was partly or entirely atelectatic. There were retractions of Shrapnell's membrane in all ears. Localized atrophy without retraction occurred either in the upper posterior or upper anterior quadrant or downward in tensa. Retraction of Shrapnell's membrane occurred in almost one-third of these ears. Atrophy localized posteriorly with retraction onto the incudostape-

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| Localized atrophy and pexi     | 1           | 1                   | 12       | 47        | 18         | 12        |                      |
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|---------------|----------------|---|---------------------|----|
|               |                |   | n                   | %  |
| DSCG          | 13             | 67  | 8                   | 45 |
| Antihistamine |                |   |                     |    |
| Phenorgan®    | 16             | 44  | 41                  | 93 |
| Tavegil®      | 16             | 44  | 41                  | 93 |
| Cortisone     |                |   |                     |    |
| Betnesol®     |                |   |                     |    |
| 1 25 000      | 17             | 31  | 6                   | 19 |
| Solu-Cortef®  |                |   |                     |    |
| 1 2 000       | 1              | 31  | 7                   | 23 |
| 1 200         | 1              | 31  | 16                  | 52 |

the reverse mixing procedure the cytotoxic reactivity of the leukocytes remained almost undiminished in 40% of the tests despite the addition of non-reactive serum. Positive leukocyte reaction was observed in 46% of tests performed with heated reactive serum (56°C for 2 h) reactive non washed cells and food antigens. Incubation with food antigens produced positive reactions in 21% of tests in which heated serum was added to washed reactive cells (Table II). Similar results were obtained when the tests were carried out with serum heated for 30 min.

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## CERVICO-OCULAR REFLEX IN THE NORMAL ADULT

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 Moss Rehabilitation Hospital Philadelphia USA

(Received April 4 1979)

**Abstract** (1) The cervico-ocular reflex (COR) in humans as measured in the subjects ( $n=10$ ) stood on a rotatable platform in a dark room with the head fixed by stationary biteplate. Eye movements were measured in response to active and passive rotations about vertical axis. (2) The COR gain (horizontal eye movement/amplitude of body rotation) was as great as 22% at low frequency of body rotation (0.025 Hz). With increasing frequency (e.g., at 0.4 Hz) the gain decreased to about 7%. (3) The phase angle of the eye movement ranged generally between  $-80$  and  $240$  degrees with an average response around  $180$  degrees. (4) During active rotation the COR response was similar to the responses measured during passive body rotation. (5) The principal conclusion is that in normal adult humans the COR does not aid in stabilization of the image on the retina during passive or active body rotations. A theoretical function for the COR is presented and discussed.

Early this century Bárány (1906) reported that he could observe reflexive eye movements in rabbits by fixing the rabbit's head and rotating its trunk about various axes. He concluded that the reflex was tonic since the eyes remained in their deviated position as long as neck torsion was maintained. Békésy & Rustin (1915) were the first to report similar reflexive eye movements in man. They described having produced compensatory eye movements in an 18-year-old male with inexcitable labyrinthitis by rotating the man's trunk while holding his head fixed. Bárány (1918) disclosed that he could produce similar eye movements in newborns and premature infants. These reflexive eye movements could only be observed during the first two days after birth after which time they became obscured by spontaneous voluntary eye movements.

The non-vestibular origin of this reflex was

recognized quite early but it was not until thirty years later that the receptor sites were convincingly shown. The strongest evidence (Biemond & deJong 1969; Hikosaka & Maeda, 1973; McCouch et al. 1951) suggests that the receptors involved with the cervico-ocular reflex (COR) reside in the intervertebral joints and ligaments of the first three cervical vertebrae.

Although cervically induced eye movements were commonly seen in experimental animals and could be observed in infants and in adults under certain pathological conditions they were never seen in the normal adult (DeKleyn & Stenvers 1941). After considerable debate over whether the reflex actually existed in normal adults its presence was finally demonstrated when researchers began testing subjects in the absence of visual feedback (Phillipszoon 1962; Phillipszoon & Bos 1963; Suzuki 1972; Takahashi & Oyster 1974). Visual feedback was commonly eliminated by placing the subject in a dark room or having him close his eyes.

Fixation of the head together with the removal of visual input allows isolation of the COR from the two other primary oculomotor reflexes—the vestibulo-ocular reflex (VOR) which responds to head acceleration (Melville Jones & Milsum, 1965) and the opto-kinetic reflex (OKR) which responds to retinal image slip (Takahashi & Oyster 1974). It has been presumed that the COR acts together with the VOR and OKR to stabilize images on the retina during active head movements (Dich-



## DISCUSSION

*Huiling to Holopainen* You investigated various aspects of the cytotoxic leucocyte reactions. Could you tell us in which kind of patients your investigations have been carried out and could you also inform us of the results of this test in normals?

*Holopainen (Reply)* Most patients with positive reactions in the cytotoxic test had nasal problems with perennial symptoms. So-called normal subjects (controls) had negative reactions or only one-two slight reactions.

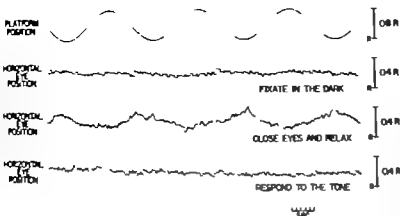


Fig. 3 Comparison between COR responses produced under three sets of instructions. Data shown are from one subject. Note that eye calibration (0.4 radians) is one-half that for the platform position.

movements have certain repeatable characteristics.

Fig. 1 contains a portion of the COR recorded from 3 different subjects. The data were selected to show the general character of neck induced eye movements. As illustrated in the figure during motionless periods (left side of figure) the subjects exhibited seemingly random eye movements around some central point. Upon the commencement of neck torsion, the baseline began to fluctuate. These "fluctuations in the baseline" will be referred to as slow phase eye movements (in opposition to the fast phase eye movements or saccades seen in nystagmus). Since typical values for gain and phase angle are given in the following section it is sufficient at this point to make two observations: first that the magnitude of the slow phase eye movements are significantly less than the magnitude of the neck movement and second that the slow phase eye movement generally occurs in the direction opposite to the direction of platform (or shoulder) movement. That is a shoulder rotation to the right (clockwise rotation when observing the subject from above) results in a small eye rotation to the left (counter-clockwise when observing the subject from above).

As demonstrated in Fig. 1 slow phase eye movements appear to sum directly with spontaneous eye movements which occur in the absence of neck torsion. The random eye move-

ments which are present before rotation begins continue on throughout the period of neck rotation.

### *Effects of stimulus frequency*

At the lower frequencies of rotation employed cervically induced eye movements are most apparent. As the frequency of neck torsion is increased the magnitude of the resulting eye movement decreases until at 0.2 Hz there is little eye movement which can be correlated with the neck torsion stimulus.

The decline in response magnitude with increasing frequency is presented in Fig. 2. This figure contains the results of the computer analysis for all 10 subjects under each of the conditions used during the passive experiments. COR response gain is less than 3% at frequencies above 0.1 Hz for the Respond to the Tone and Fixate in the Dark instructions. The largest response is seen at the lowest frequency examined 0.025 Hz. At this frequency the maximum mean gain observed was approximately 22%.

The phase of the COR response generally ranged between  $-80^\circ$  to  $-240^\circ$  as seen in Fig. 2. There appears to be a slight increase in phase lag with increasing frequency of rotation although average phase angle response ranges around  $-180^\circ$ . The significance of this fact to ocular stability will be presented in the discussion.

## DISCUSSION

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*Holopainen (Reply)* Most patients with positive reactions in the cytotoxic test had nasal problems with perennial symptoms. So-called normal subjects (controls) had negative reactions or only one-two slight reactions.

rotation. An assumption has been made here that neck receptors will be stimulated equally well by a body rotation (head fixed) as by a head rotation (body fixed). However, the direction of rotation would be opposite. That is, head rotation to the right (body fixed) would be equivalent to a body rotation to the left (head fixed). Therefore, an eye movement which would aid in eye stabilization (as does the VOR) would be in the same direction as the body movement—a phase angle of  $0^\circ$  between the eye and body movements. In general, then, since eye movement and body movement were  $180^\circ$  out of phase rather than reducing retinal image slip, the COR response enhanced it. A similar finding was reported by Gesty (1976) working with rabbits.

What then is the function of the cervico-ocular reflex? A speculative answer to this question is suggested as follows. The vestibulo-ocular reflex provides an image of head acceleration to the oculomotor centers to help in eye fixation during head movement. It is proposed that the COR provides an image of body movement to the oculomotor centers. The need for separate head image and body image to provide eye fixation becomes apparent during complex movements of head and body. For example, assume that the head is moving to the left at  $5^\circ/\text{sec}$  and the body is also moving to the left but with a velocity of  $70^\circ/\text{sec}$ . In this case, the VOR would produce an eye movement toward the right (responding to the  $5^\circ/\text{sec}$  head rate); the COR would sense the relative neck torsion to the left ( $1^\circ/\text{sec}$  rate) and would produce an eye movement also to the right. The combined eye movement produced by VOR and COR would therefore tend to reduce retinal image slip.

Despite the fact that the summation of VOR and COR effects will not always occur as it does in the example, the concept of an eye movement opposed to head motion (VOR) and an eye movement opposed to body movement (COR) is satisfying. Although such a function for the COR is speculative, it suggests the path to experimental validation.

## ACKNOWLEDGMENTS

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## ZUSAMMENFASSUNG

1. Der cervico-okuläre Reflex (COR) beim Menschen wurde gemessen, während die Versuchspersonen (Zahl 10) auf einer Drehtafel standen. Der Kopf der Versuchsperson wurde von einer stationären Beißplatte festgehalten. Die Augenbewegungen wurden als Reaktion der aktiven und passiven Körperrotation um einer vertikalen Achse gemessen.

2. Der Verstärkungsfaktor des COR (d.h. horizontale Augenbewegung/Schwingungsbreite der Körperrotation) war so stark als 22% bei einer schwachen Frequenz der Körperrotation (0,025 Hz). Bei zunehmender Frequenz (z.B. 0,4 Hz) nahm der Verstärkungsfaktor bis zu 5% ab.

3. Der Phasenwinkel der Augenbewegung lag in den meisten Fällen zwischen  $-80^\circ$  und  $-40^\circ$  Grad mit einer durchschnittlichen Reaktion von ungefähr  $-180^\circ$  Grad.

4. Während der aktiven Rotation war der COR den Reaktionen der passiven Körperrotation ähnlich.

5. Das Hauptergebnis ist folgendes: Beim normalen wachen Menschen unterstützt nicht der COR die Stabilisierung von dem Erscheinungsbild auf der Netzhaut während der passiven oder aktiven Körperrotationen. Eine theoretische Funktion des COR wird vorgelegt und diskutiert.

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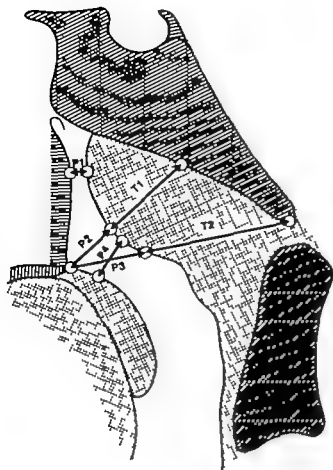


Fig 1 Radiographic measures of the nasopharyngeal airway

adenoid size and adenoidectomy is generally performed according to clinical evaluation of the children. A history of nasal obstruction and snoring is the most common indication for surgical intervention but Hibbert & Whitehouse (1978) state that preoperative signs and symptoms are poor predictors of the adenoid weight and that radiography is a more accurate method as a basis for surgery.

The purpose of the present study was to evaluate the relationships between (1) radiography (2) rhinomanometry and (3) clinical symptoms and signs in adenoid children in order to reach a more accurate assessment of the nasopharyngeal airway in each patient.

## MATERIAL AND METHOD

Twenty-four children aged 4.7–12.5 years, mean age 8.7 years, admitted to hospital for adenoidectomy were studied. They all sub-

sequently underwent adenoidectomy and were controlled 2 months later.

A history of symptoms was taken and especially the following symptoms were recorded: (1) Recurrent Upper Airway Infection (RUAI) (2) Snoring (3) Rhinorrhoea (4) Mouthbreathing.

All children underwent a clinical examination by an otologist involving anterior and posterior rhinoscopy as well as inspection of ears, nose and throat. No child suffered from upper airway infection at the time of examination.

## Radiography

Radiological examination was performed immediately after the clinical evaluation. Lateral as well as postero-anterior radiographs were taken using a Lumex cephalostat. In the lateral radiographs the central ray passed through the two earrods of the cephalostat which had been inserted in the external ear canals. The head was thereby positioned so that the median plane was parallel to the film. With the focus median plane distance of 180 cm and the film-median plane distance of 10 cm the enlargement of the lateral films was 5.6%.

The following measurements were made on the lateral radiographs (Fig. 1):

### 1 Size of the airway

P<sub>1</sub> The shortest distance from the most anterior part of the adenoidal mass to the posterior wall of the maxillary antrum (Hibbert & Whitehouse 1978).

P<sub>2</sub> The distance from the pterygomaxillary point (the intersection of the contour of the nasal floor with the posterior contour of the maxilla) to the adenoid tissue along the line from the pterygomaxillary point to the mid point of a line joining basion (the most posterior inferior point on the clivus of the occipital bone) and the center of sella turcica (Linder Aronson & Henrikson 1973).

P<sub>3</sub> The distance from the pterygomaxillary point to the posterior pharyngeal wall along the line from the pterygomaxillary point to basion (Linder Aronson & Henrikson 1973).

Table II Relationships between VS of caloric nystagmus and lesion site

| Lesion site | Loss of VS | VS |
|-------------|------------|----|
| IO          |            |    |
| rostral     | 0          | 4  |
| caudal      | 0          | 3  |
| SC          | 7          | 2  |

abnormality of OKN OKN-DP to the left alone falls in the lower right corner (shaded area) and OKN DP to the right alone in the upper left corner (shaded area). The other three areas signify bilateral OKN diminution (lower left square) and unidirectional diminution of OK responses combined with OKN DP. The lowest and highest nystagmus beats were 33 and 153 which are indicated by horizontal and vertical dotted lines respectively (Fig. 1). Thus the normal range fell in the hexagonal area. After the follow-up studies were completed the brain of the animal was perfused with 10% formal-saline solution through the left cardiac ventricle. The extent

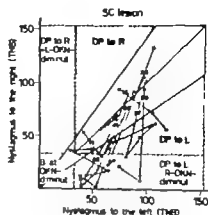


Fig. 3 Optokinetic diagram using TNB as parameter OKN in cats with SC lesions tends to show OKN-DP to the left with diminished OK responses to the right except for 2 cats (14, 15) belonging to Group I.

of lesions was determined by serial sections of paraffin-embedded specimens and reproduced by a projecting device.

## RESULTS

Of 42 cats investigated 30 survived the electrolytic lesions of the IO or the SC. In 25 of the 30 cats, the control test could be performed thoroughly before and after lesions were made. In 7 of 25 cats the lesions were found outside the target area. The data for those 7 cats were therefore discarded ultimately. Table II summarizes the relationships between VS of caloric nystagmus and the lesion site. VS of caloric nystagmus was revealed in all the IO-lesioned cats. With the SC lesion VS was not recognized in 7 of 9 cats. OKN investigated on these cats is described according to lesion site.

### IO lesion

In 4 of 9 cats lesions of the IO were found to cover the rostral part, but not the caudal part of the medial accessory olive (MAO) and in the remaining 5 cats lesions completely covered the dorsal cap of the MAO. OKN in the IO-lesioned cats were plotted in the optokinetic diagram. A normal range of TNB in 9 cats fell in

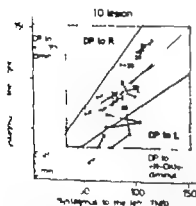


Fig. 2 Optokinetic diagram using TNB as parameter in the IO-lesioned cats. The first open circle in the hexagonal area indicates TNB before IO lesions. The subsequent changes of OKN according to time course are indicated with arrows. Each arrow is interrupted with the cat number. About week intervals exist between the one end of an arrow and the other end for an open circle or square and a filled circle. The OKN in all the IO-lesioned cats are unaffected and every open square falls within the normal range except for cat no. 35.

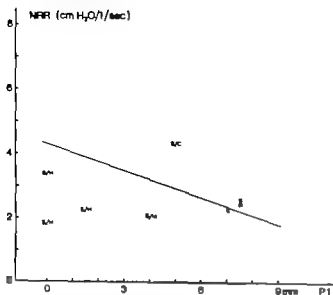


Fig 2 Association between nasal respiratory resistance (NRR) and nasopharyngeal airway at P ( $r=-0.52$ ,  $p<0.05$ ). Letters indicate main symptoms. S=Snoring H=Hearing loss C=RUA

sue measured at  $T_1$  was also correlated with NRR ( $r=0.58$   $p<0.01$  Fig 3) whereas the correlation between  $T_2$  and NRR was not significant ( $r=0.37$ ).

The relationships between the symptoms are shown in Table III. Probably significant correlations ( $p<0.05$ ) were found between RUA and Rhinolalia and between Snoring and Mouthbreathing.

The relationship between the radiographic and rhinomanometric recordings and the symptoms are given in Table IV. All symptoms showed correlations of about 0.3 with NRR but these were not significant with the present sample size. The nasopharyngeal airway measured by the radiography was negatively correlated to the symptom Snoring ( $p<0.05$  at  $P_1$  and  $P_4$ ). This is in agreement with the findings of Hibbert & Tweedie (1977). Mouthbreathing showed negative but non-significant correlations with  $P_1$ – $P_4$ .

The depth of soft tissue measured at  $T_1$  and  $T_2$  showed no significant correlation to any symptom but the highest correlations ( $r=0.4$ ) appeared with the symptom Snoring (Table V).

Measurement of the width of the nasal airway on the posteroanterior radiographs

showed no correlations with radiographic rhinomanometric figures.

The semiquantitative evaluation of amount of surgically removed adenoid tissue showed no relationship to any other parameter.

## DISCUSSION

Radiographic assessment of the nasopharyngeal airway can be performed with great accuracy and reproducibility when carried out under well-defined conditions and cephalostatic fixation of the head.

Recording of nasal respiratory resistance NRR has previously been reported possible only 50% to 75% of the cases but is possible in all subjects down to about 5 years of age by means of a specially developed technique involving individual fitting of a mask and bio feedback monitoring of the recordings (Solow & Greve 1980).

By means of the above techniques the present study aimed to examine in detail the relationships between radiographic and rhinomanometric measures of the nasopharyngeal airway and some clinical symptoms of airway inadequacy.

The highest correlation between the nas

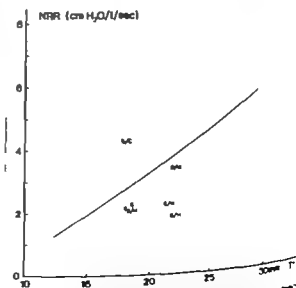


Fig 3 Association between nasal respiratory resistance (NRR) and adenoidal prominence at T ( $r=0.58$   $p<0.01$ ). Letters indicate main symptoms. S=Snoring H=Hearing loss, C=RUA

unde zu 100 Katzen OKN nach Läsion der unteren Olive und des Colliculus superior wodurch der Flocculus das visuelle Signal empfangt, untersucht. Nach Läsion der unteren Olive wurde die visuell ausgelöste Suppression des kalorischen Nystagmus immer noch beobachtet und OKN abgesehen von einer Katze auch normal ausgefallen. Nach Colliculusläsion wurde der Effekt des Lichts auf den kalorischen Nystagmus an 7 von 9 Katzen nicht beobachtet und OKN in diesen Katzen zeigte das Ruckungsüberwiegen nach links und die Verminderung oder das Ausfall nach rechts. Von diesem Resultat darf man daher wohl sagen, daß Colliculus superior als präfloccularer Kern eine wichtige Rolle spielt.

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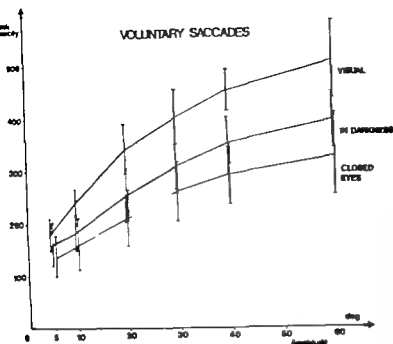


Fig. 3. Mean peak velocities with standard deviations of voluntary saccades when performed in visual conditions, in darkness and in darkness behind closed eyelids at different amplitudes.

At 20° amplitudes the mean peak velocity of the saccades in light was about 340°/s, in darkness about 250°/s, and behind closed eyes about 210°/s (Fig. 3). From these values the peak velocities increased with increasing amplitudes of the saccades and at amplitudes of 60° were 500°/s, 400°/s and 320°/s respectively (Fig. 3).

Saccades were characterized by highly variable velocities when performed without visual control. Sometimes efforts to produce saccadic eye movements with closed eyes resulted in recordings appearing more as slow deviations of the eyes than as fast saccadic eye movements (Fig. 4). In darkness, overshooting saccades were very common especially at the amplitudes of 10° and 20°.

There was further a considerable inter individual variation in saccadic velocity. Since this study was focussed on the mean values of a whole group of subjects examined in standardized visual conditions no further interest was paid to either inter or intrasubject variations.

We found no difference in the peak ve-

locities of saccades between different age groups (Fig. 5). Young subjects had no higher saccadic velocity than older ones. In fact a subject aged 72 had faster saccades than another subject aged 22.

The rapid return eye movements following the pursuit eye movements were analyzed in five subjects. These rapid eye movements had the same amplitude-velocity relationship as visual saccades. Hence we considered these movements identical to the saccadic ones.

*Fast phases of nystagmus* We found no difference in the peak velocities of the fast phases of caloric nystagmus, whether induced by irrigation of the right or of the left ear by cold or by warm water. These peak velocities were as were the velocities of the saccades strongly dependent upon the amplitude of nystagmus as shown in Fig. 6. Moreover the fast phases of nystagmus were equal whether caused by rotation or by caloric stimulation. The peak velocities of the fast phases of nystagmus induced by rotation at amplitudes of 20° were about

caused by excessive adenoidal tissue and that cephalometric radiography and rhinomanometry may provide valuable information for the clinical diagnosis of this condition

## ZUSAMMENFASSUNG

An 74 Kindern die an unsere Abteilung zur Adenoidotomie gewiesen wurden nahm man auf Grund von subjektiven Symptomen Radiographie und Rhinomanometrie eine Beurteilung der nasopharyngealen Luftwege vor. Schnarchen war das einzige wesentliche Symptom das auf der Einengung des Nasopharynx in Verbindung gesetzt werden konnte. Die nasale Atmungsresistenz stellte man in Relation zu den radiographischen Messungen der Luftwege und den Tiefenmessungen der Weichteile der hinteren Pharynxwand welche mittels einer neuen Technik gemessen wurde. Dadurch konnten auch Kinder bis zu einem Alter von nur 5 Jahren rhinomanometrisch beurteilt werden. Sowohl die Radiographie als auch die Rhinometrie sind wertvolle Parameter für die Indikation zur Adenoidotomie.

## ACKNOWLEDGEMENTS

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## DISCUSSION

Tonndorf: Sørensen Question: What breathing rate did you use?

You showed us that there was no correlation between tissue mass and the breathing impairment. The most prominent factor with an adenoid mass is the Bernoulli effect: i.e. the sucking in of the soft wall, which is a function of flow velocity. I would therefore propose that you increase the breathing rate until flow resistance increases. You will then have another useful measure.

Sørensen (Reply)

In the examination we wanted the children to breathe in their natural way in order not to force either the rate or the pressure. In this way we obtained the best reproducibility of the recordings in each child.

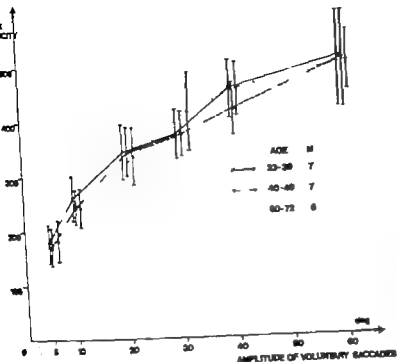


Fig 5 Mean peak velocities with standard deviations of visual voluntary saccades in three different age groups at different amplitudes.

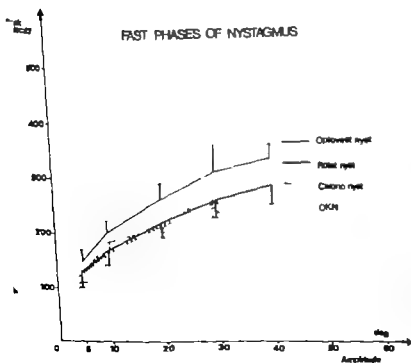


Fig 6 Mean peak velocities and standard deviations of fast phases of optokinetic nystagmus (—), of rotational nystagmus (---), of caloric nystagmus (· · ·) and of optometric nystagmus (—). The spreads are presented only in direction

caused by excessive adenoidal tissue and that cephalometric radiography and rhinomanometry may provide valuable information for the clinical diagnosis of this condition

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Sørensen (Reply)

In the examination we wanted the children to breathe in their natural way in order not to force either the rate or the pressure. In this way we obtained the best reproducibility of the recordings in each child.

ported the idea that all types of rapid eye movements should be equally rapid. Our observations to the contrary may have some support in previous literature also.

Thus the normal contraction of the antagonistic muscles present only during the fast phase of nystagmus (Shimazu 1972) might explain a difference in velocity between fast phases and saccades. Further the "slow" saccadic velocities noticed e.g. in Huntington's chorea, has been believed to be due to a lack of proper inhibition of antagonistic muscles (Starr 1967).

One other factor which seems to be of importance is that saccades are initiated voluntarily but fast phases of nystagmus are released automatically and secondarily to slow phases. During voluntary saccades the subject is alert and active performing a task while during fast phases of nystagmus the subject is passive. The higher velocity of the saccades might therefore at least in part be explained by a more powerful excitation of pontine neurons caused by volition or related to alertness caused by this volition.

## ZUSAMMENFASSUNG

Willkürliche schnelle Augenbewegungen (Saccaden) und reflexbedingte schnelle Augenbewegungen (schnelle Nystagmusphasen) wurden bei 20 gesunden Personen miteinander und untereinander verglichen. Die Geschwindigkeiten beider Augenbewegungstypen stiegen mit einer Zunahme der Amplituden an. Es wurde gefunden, daß die willkürlichen Augenbewegungen, die Saccaden, um Licht am schnellsten, langamer im Dunkeln und am langsamsten hinter geschlossenen Augenlidern and während der Geschwindigkeiten der schnellen Phasen von optokinetischen und vestibulären Nystagmen identisch waren, erreichten die schnellen Phasen von opto-vestibulären Nystagmen (sowohl optische als auch vestibuläre Stimulation) signifikant höhere Geschwindigkeiten als beide anderen Typen. Bei gleicher Amplitude und unter gleichen visuellen Bedingungen waren die Geschwindigkeiten der willkürlichen schnellen Augenbewegungen (die Saccaden) signifikant höher als die schnellen Komponenten reflexbedingter Augenbewegungen.

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Fig 1 Cholesterol granuloma—the fluorescent (i.e. the bright spotty areas) denotes the presence of lactoferrin

H One biopsy from a tympanosclerotic region

All biopsies were also examined for control with fluorescent IgA but without antilactoferrin

### RESULTS

- 1 The 10 mucus samples were strongly positive showing fluorescent anti IgG binding to anti lactoferrin denoting the presence of lactoferrin
- 2 Biopsies from normal mucosa showed a faintly positive fluorescent reaction in one and hardly any reaction in the two others
- 3 The 17 biopsies from the dry mucosa of simple chronic otitis media were positive but not strongly so
- 4 All 15 biopsies from cholesterol granulomas (Fig 1) the 45 biopsies from simple wet chronic otitis media—and the 20 biopsies from cholesteatomatous ears—were strongly positive. This was also the case with the salivary glands
- 5 All controls with fluorescent anti IgG in the absence of anti-lactoferrin washed away and gave negative results
- 6 All controls of tissues without lactoferrin (i.e. muscle connective tissue) were fluorescent negative

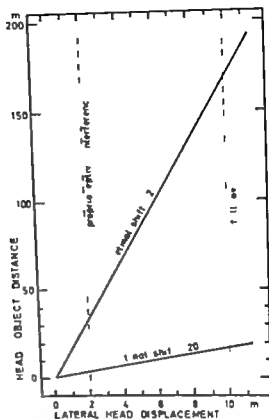
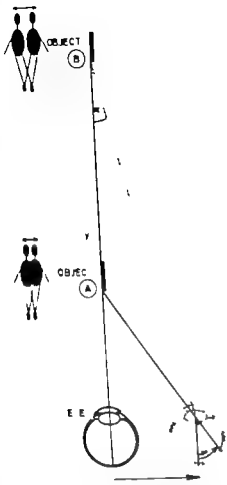
In summary (A) All secreting mucosae were

positive for lactoferrin. The more infected the mucosa (the more mucus it contains) the stronger the fluorescent reaction denoting a higher content of lactoferrin. (B) The mastoid areas (which contain fewer mucus secreting elements) were less positive than the middle ear proper. (C) All controls were negative.

### DISCUSSION

A blue (steel blue) tympanic membrane can hide behind it a true post traumatic haemotympanum, a bulging jugular bulb or a chondroma. But the most common of all is the "Idiopathic haemotympanum" called The Blue Eardrum, which appears in prolonged secretory otitis media; its colour has probably little if anything to do with the presence of the few red blood corpuscles found on microscopic examination behind the tympanic membrane—in spite of its name. Fresh blood or microscopic amounts of blood are not found in these tympanic cavities.

The Blue Drum is characterized by a long standing and chronic history of S O M, a fact which has already been recognized by Paparella & Lim (1967) and Sheehy et al (1969). This we would like to confirm as we have seen ears which started as banal S O M, turn blue after 6–10 years—and even less



Geometrical analysis indicates that in order to be easily detected, body sway must increase with increasing distance between the eyes and the nearest stationary objects. Angular displacements  $\alpha$  on the retina, caused by lateral head displacement, are smaller the further the distance to the object. Diagram shows rela-

tionship between head-object distance  $y$  and lateral head displacement, for given retinal displacement thresholds of either 2 or 20 minutes of arc. However, because postural regulation involves multiloop control, additional proprioceptive cues may alter sway amplitudes as well.

(1955) a normal lateral head sway of 2 cm would be subthreshold at a distance of about 3 m. This should lead to a perceptual conflict as the vestibular and somatosensory receptors sense a body shift which the visual system cannot detect. The conflict might be resolved by increasing the postural sway and thereby reactivating visual control. For a simple loop control a relationship between distance and sway amplitude could be expected ( $\tan \alpha = y$ ) with the gain dependent on the retinal movement detection threshold. However

with the lower limbs close together at free stance one falls over at a head sway more than 10 cm. Thus at an eye-object distance of 15–20 m a maximal body sway would produce retinal angular deviations ( $\alpha$ ) less than 20 mm of arc according to the trigonometrical model. Since we are dealing with a multiloop control of postural balance it can be assumed that with increasing sway amplitudes the particular sensory weight of the somatosensory and vestibular afferences becomes greater increasing their contribution in pos-





Fig 3 Higher magnification of a cholesterol granuloma stained with Prussian Blue—dark spots are positive stain for iron. Arrow—cholesterol crystals

so successful in producing cholesterol granulomas experimentally including iron deposits in mastoid cells of squirrel monkeys by obstructing the Eustachian tube for 6 to 12 months. Their micrographs show black areas which they interpret as the presence of a hemorrhage.

The findings of all these experiments reaffirmed that when a cavity which usually contains air (in this case the ear) is chronically underaerated an inflammatory granuloma with deposits of cholesterol crystals will appear. The cholesterol crystals are either synthesized *in situ* or transported from elsewhere and deposited there. The biological significance of the appearance of cholesterol granulomas is not clear. The classical notion that cholesterol crystals are witness to a previous haemorrhage needs proof, however. This notion might be a carryover from days gone by which was not substantiated in the first place.

Chronically underaerated middle ear clefts pass through episodes of variable negative pressure. It is this negative pressure which is postulated as bringing about blood vessel lesions, extravasation of blood which will show as a blue drum and lead to hemosiderin deposits. This is in a sense an extension of the classical *ex vacuo* theory. However the

negative pressure in the clinical cases is in all probability not abrupt enough to cause rupture of blood vessels as the case is when the Eustachian tube is clamped shut in experimental occlusions. There is also usually no histological evidence of bleeding blood vessels associated with such an event in ears with cholesterol granulomas. It might therefore be more realistic to regard the red blood cells found in the vicinity of cholesterol granulomas as the consequence of surgery and look for another explanation for the presence of iron deposits. An alternative explanation to the presence of iron deposits can be found in our detection of lactoferrin which is an iron-chelating agent. We have found lactoferrin to be present in all ears where cholesterol granulomas appear—and none in controls. The explanation for the presence of cholesterol crystals eludes us so far—synthesis *in situ* due to the change environment is one possibility.

Lactoferrin was originally detected in milk—hence its name (So & Sorensen 1939). Later lactoferrin was found to be present in all exocrine secretory organs (Afassod

Actually already in 1971 was cholesterol described by Van de Calseide et al (*Acta Otolaryng* 71: 153–158, 1971) in mucus from secretory otitis media and this could well be the source.

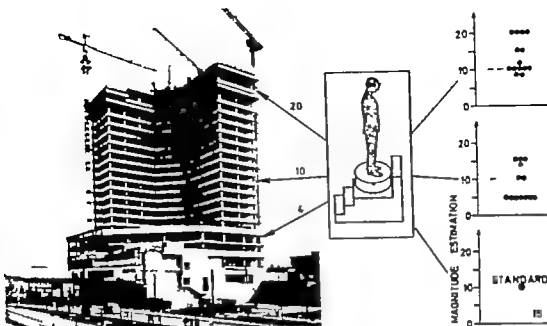


Fig. 5. Saturation of subjective height vertigo at increasing altitudes (20, 50 and 100 metres: 4th, 10th, and 20th floor respectively). Magnitude estimations of 15 subjects

were not significantly different for the three altitudes. The fourth floor (20 metres) served as the standard situation (modulus with an arbitrary value of 10)

desired perceptual cues provided by windows and visible ceilings were prevented by restriction of peripheral vision to a total field of 120 degrees in diameter. Subjects were blindfolded and taken in an outside elevator to the fourth floor at an altitude of approximately 20 m. Thereupon via a staircase they stepped onto the stabilometer platform which stood adjacent to the outer wall of the building. The blindfolds were removed and the subjects suddenly exposed to the height vertigo inducing situation during which they were required to look down for a period of one minute. A security belt was used as a precautionary measure. The fourth floor condition served as the standard situation for further magnitude estimations of subjective vertigo (modulus with an arbitrary value of 10). Posturographic measurements were performed of the fore-aft and lateral body sway with eyes closed and eyes open as well as obtaining the subject's scalings of height vertigo severity. All's were then taken from the fourth to the twentieth and finally to the tenth floor representing an alti-

tude of about 100 and 50 m respectively. Technical factors within the building site prevented randomization of the order of exposure to the different heights.

As depicted in Fig. 5 statistically there was no significant difference among the height vertigo intensities experienced at altitudes of 20, 50 or 100 m, although some S's gave greater estimations on the twentieth floor. For most subjects vertigo was already maximal on the fourth floor. Thus subjective height vertigo seems to be saturated and maximal at a height of about 20 m. Habituation may have contaminated the data, however. This is suggested by the slight decrease of the mean scaling value at floor 10 compared with the standard at floor 4.

The stabilograms of postural sway taken under these natural environmental conditions were affected by occasional wind and an extensive body tremor due to acrophobia (Fig. 6). This tremor had a frequency of 5–10 Hz and did not represent the visually induced sway of height vertigo because it continued

That lactoferrin is present in excess in middle ear granulomas could be taken as the mucosa's means of self-defence. Why middle ear under aeration by itself should promote the local appearance (synthesis) of lactoferrin or indeed of cholesterol crystals is not known.

## ACKNOWLEDGEMENT

This work was supported in part by a NIH grant no NS-10048-08.

## ZUSAMMENFASSUNG

Direkte manometrische Messungen des Gasdruckes im Mittelohr wurden durch ein in der tympanischen Membran eigens mit hohler Nadel gemachtes Loch gasverlustlos durchgeführt. Die so gemessenen jedoch kleine negative Drücke (ein paar mm von  $H_2O$ ) im Falle von S.O.M. und Atelektase sind aber falsch, da ein sehr grosser bis jetzt unerachteter Korrekturfaktor angewendet werden muss. Für diese Korrektur muss man aber das Volumen des freien Gasraumes des bestimmten Mittelohres genau kennen. Obwohl dieses Volumen im Einzel fall uns unbekannt war, ist es gut bestätigt, dass das Mittelohrvolumen in diesen Fällen zwischen 0,5 und 1 ml liegt. Eine mittlere Grösse von 1,2 ml wurde darum angenommen und für alle Korrekturen verwendet. Unsere so korrigierten Resultate waren  $-34 \pm 86$  mm von  $H_2O$  für 4 Fälle von S.O.M. und  $-9 \pm 86$  mm von  $H_2O$  für 32 Fälle von Atelektase. Diese Messungen wie schon solche von anderen, zeigen eine sehr breite Streuung. Dafür kann man zwei Gründe angeben: (1) Dass mit einem Mittelwert und nicht mit dem genauen Mittelohrvolumen des bestimmten Ohres gerechnet wurde. (2) Dass wirkliche Schwankungen der Mittelohrdurchblutung und deshalb des Druckes vorliegen. Der Luftungsangel im Ohre ist deshalb eine funktionelle Störung und keine Blockung des Kanals des Eustachius.

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tip does not occur on the ground despite the fact that visual targets may be at a great distance because cues from nearby stationary contrasts (provided by peripheral vision) prevent the instigating sensory mismatch.

This might become different if the ground is not aligned to the earth horizontal although the relative influence of the angle of incline/decline has not been studied thoroughly. In preliminary experiments we found (with great intra- and inter-subject variability) that for a given altitude subjective height vertigo can be induced at an angle of decline of 45–50 degrees and that it progressively increases with increasing slope up to a maximal vertigo. With increasing slope peripheral vision provides less information about nearby stationary contrasts. According to posturographic measurements the development of subjective height vertigo also requires an exposure time of 5 to 30 sec to be saturated.

All visual stimulus characteristics which have an influence on the occurrence and intensity of physiological height vertigo concomitantly affect postural balance. Purkinje (1820) was the first to attribute height vertigo symptoms to a postural imbalance of upright station (he stressed the unusual and therefore unadapted stimulation at gaze downward with the stationary visual reference in an imaginary space below foot support) as later was assumed more concretely by Lee & Lishmann (1975), Brandt (1976), Bles et al. (1978) and Brandt et al. (1978). Purkinje (1820) and Kobrak (1974) erroneously concluded as have modern textbooks that height vertigo mainly represented a disorder in neurosis or psychiatric patients.

Pogány's (1958) finding, that patients with vestibular dysfunction exhibited a greater susceptibility to height vertigo is now understandable since the erroneous visual signal should have a disproportionately greater sensorial weight when associated with a vestibular lesion. This is analogous to our experiments (paper II Bles et al. 1979) where induced somatosensory or vestibular systems

disturbances (e.g. when standing on foam rubber or being carried in a quivering open elevator attached to the outside wall of a building under construction) enhanced subjective height vertigo symptoms.

### *Physical prevention of height vertigo*

Knowledge of the stimulus characteristics required for the optimal visual contribution to stabilized postural balance also dictates practical advice related to the reduction of height vertigo in susceptible subjects.

1. One should avoid the free upright stance in critical situations at high altitudes. This is done intuitively when grasping for stationary framework or leaning against a wall for support.

2. When looking down, one should obtain stationary cues from nearby contrasts in the peripheral visual field. (Visual stabilization of posture is served primarily by the retinal periphery while the central retina mainly serves egocentric recognition and pursuit of objects.) Staring at moving objects such as clouds increases the danger of falling because additional postural destabilization through linearvection may be induced. One should avoid long exposure times as height vertigo usually has a latency taking several seconds to develop. Looking through binoculars is very dangerous because it restricts the visual field and introduces the unusual and therefore unadapted magnification factor.

3. Body and head position should be adjusted to the gravitational vector because vision will receive a relatively greater sensorial weight (which is undesirable) if the otoliths are displaced beyond their optimal working range by extreme head tilt. It may also be true on the basis of other observations that the feet should be firmly planted on an earth horizontal surface.

### *Physiological height vertigo versus acrophobia*

Physiological height vertigo must be differentiated from neurotic acrophobia or height

## EXPERIMENTAL AGEUSIA

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University of Granada, Granada, Spain*

**Abstract** By administering acetyl-homocysteine and D-Penicillamine to the guinea pig, ageusia and hypogeusia respectively were obtained. Copper concentration was estimated in serum, saliva, liver, salivary glands, muscle, heart and tongue. A significant decrease in the concentration of this metal in the liver was observed and a slight increase in the rest (average values). The results were similar for both drugs. We believe that copper does not play any role in the taste process. The fact that this and other metals restore taste would indicate that those metals bind the SH groups of the drugs and that they are probably responsible for the ageusia.

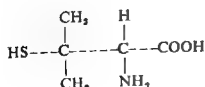
It is known from clinical experience that the administration of thiols reduces taste acuity. This has been observed by us in some patients suffering from chronic pharyngitis and to whom acetyl-homocysteine was administered. On the other hand, patients treated with D-Penicillamine frequently complain of decreased taste sensitivity. Henkin et al (1967) were the first to observe ageusia in different conditions treated with this drug.

Considering that D-Penicillamine is a copper-chelating agent which depletes copper, it was assumed that this metal was crucial for taste sensitivity. It was thought that the ageusia observed in those cases was attributable to the lack of copper. D-Penicillamine, however, is also a thiol contributor by one of its SH groups, as also is homocysteine.

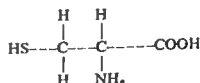
Clinical and experimental experience has shown that taste acuity returns to a normal level following the administration of copper to individuals treated with these drugs (Henkin et al 1969). However, other transition metals such as zinc and nickel achieved the same effect.

D-Penicillamine and Acetyl-homocysteine are drugs with similar chemical structures; both are amino acids and both contain thiol groups (Scheme 1).

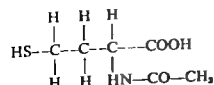
It is possible to explain the action of D-Penicillamine upon taste sensitivity by

D-Penicillamine ( $\beta\beta$  dimethylcysteine)

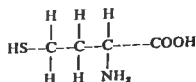
Cysteine



Acetyl homocysteine



Homo-cysteine



Scheme 1

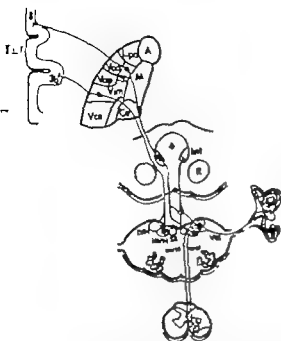


Fig. 1. Diagram showing the main ascending and descending vestibular efferents. The demonstration of these nuclei corresponds to Hassler's nomenclature (LVN & MVN: lateral and medial vestibular nuclei, and LVN & MVN: lateral and medial vestibulospinal tracts).

with the different parts of the vestibular nuclear complex. From Lorente de Nó's observations (1938) we know that semicircular canals project chiefly to the superior and medial nuclei, whereas the utricle supplies the lateral nucleus (or Donders' nucleus) (Fig. 1).

The specific stimulus for the semicircular canals consists in angular acceleration or deceleration of the head which activates the ampullar cristae during movements of the head. The result of this is the nystagmic reaction characterized by slow conjugated deviation of the eyes in the plane of the canal and counter to the direction of movement (slow phase) with a rapid return to the previous position (fast phase). Thus the semicircular canals that Brodal calls kinetic labyrinth records angular movements of the head. This is in agreement with the anatomical data, since the medial vestibular nucleus that receives the

impulses from the ampullar cristae projects in turn to the oculomotor nuclei (via the fasciculus longitudinalis medialis) and to the cervical and upper thoracic spinal cord (via the medial vestibulo-spinal tract).

On the other hand the specific stimulus for the hair cells of the utricular macula is the pressure of the otoliths over the particular region where they rest according to the varying attitudes of the head. The utricle—and probably the saccule too—constitute the static or tonic labyrinth. Its impulses are transmitted mainly to the lateral vestibular nucleus of Donders and thence to the lumbar and sacral spinal cord (via the lateral vestibulo-spinal tract).

The lateral vestibulo-spinal tract terminates entirely according to histological studies of Nyberg-Hansen in the ipsilateral laminae VII and VIII of Rexed of the lumbosacral spinal cord. Laminae VII and VIII contain exclusively the soma of interneuron that of motoneuron being located in lamina IX. However the dendrites of the latter extend to far distant points in laminae VII and VIII (Ramon y Cajal, L. de Nô, Scheibel & Scheibel). There is thus histological data suggesting monosynaptic axodendritic connections between vestibulo-spinal fibres and motoneurons as well as axo-somatic contact with interneurons. Since the work of Lund & Pompeiano in 1965 various authors have provided further physiological evidence in support of this view. From these studies it appears that the descending lateral vestibulo-spinal fibre ends monosynaptically in  $\alpha$ -motoneurons (Grillner et al. Shapovalov, Sherrington),  $\gamma$ -motoneurons (Grillner et al. Carlsson et al., Pompeiano et al.) and interneurons (Grillner et al.). Grillner in 1972 succeeded in provoking monosynaptic EPSPs in ipsilateral  $\alpha$  and  $\gamma$ -motoneurons of extensor nucleus of the ankle following stimulation of Donders' nucleus. Only occasionally can monosynaptic EPSPs be recorded in ipsilateral extensor of the knee. Indirect evidence suggests that their effects are exerted almost exclusively on static  $\gamma$ -motoneurons (S. Grillner). Disynaptic

those to which copper is more or less tightly bound. Ceruloplasmine is the best known but actually many others exist in various tissues and organs. In view of these facts we investigated copper in sensory tongue mucosa, saliva and salivary gland since they constitute the organ of taste and the fluid and organs having a close relationship to sense of taste.

A light microscope study of the taste buds complemented this part.

Second. An ultrastructural study of the ageusia taste buds in order to demonstrate what was the level at which these substances actually act. This part will be the subject of a second article because in most cases we used the tongues of our series for copper determination purposes.

## MATERIAL AND METHOD

Sixty-five guinea pigs divided into three groups were studied. Two groups of twenty-five animals each were treated with Acetyl-homocysteine and D-Penicillamine respectively until ageusia or hypogeusia appeared. A third group of 15 animals was used as a control. The weight of the animals was between 150 and 300 g.

Five animals of each group were used for macroscopic and light microscope study. The remainder were used for copper determinations.

Blood, serum, muscle (masseter), salivary gland (submandibular), heart and liver were obtained at necropsy as well as tongue mucosa. As regards the removing of the tongue and the obtaining of the saliva sample we have to be a little more explicit. In so far as the tongues are concerned we knew previously the exact location of the papillae in guinea pig. They have two large foliated and 20 to 30 fungiform ones widespread along the base of the tongue. By using osmium tetroxide they appear dense black so we easily ascertained which was the part of the tongue that we have to remove in the future without any

staining. When the animals were killed by decapitation the tongue was removed and we peeled off the mucous membrane containing the papillae using a stereoscopic microscope.

Saliva was obtained by suction with a pipette previously washing the mouth with distilled water and rejecting the first drops. Sometimes we instilled some drops of lemon juice in order to stimulate the secretion but in most cases this was not necessary.

At the beginning of the experiment we examined the taste in each animal as follows. The animal was kept for 2 days without drinking then we offered them five discs with water each with one of the four qualities dissolved in it (saccharose, sodium chloride, citric acid and quinine chloride) and the fifth one containing fresh water. The animals immediately selected the correct disc to drink. This method was also used when testing the animals submitted to the drugs. However after some experience we came to the conclusion that it was sufficient to use only one tastant because the hypogeusia was always for the four qualities. We chose as a standard substance test 0.035 quinine chloride. However in many cases it was necessary to use the complete test.

200 milligrams per kg and day of Acetyl-homocysteine in a single intraperitoneal dose was administered to the animals of the first group and to the second 28 mg/kg of D-Penicillamine by the same method.

Taste was examined following the method described every 7 days and the animals were killed when ageusia was reached. This generally occurred between day 20 and day 30. However in the D-Penicillamine group we could never obtain an ageusia state but only light hypogeusia and even then not in all the cases. When we increased the dosage most of the animals died and the same happened when we prolonged the administration time for more than a month. Consequently we decided to kill them as soon as hypogeusia appeared. In these cases we examined the taste using a series of solutions with decreasing concentration.

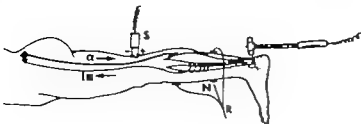


Fig 3 The position of the recording and stimulating electrodes as well as the pathways involved in the vestibular reflex are represented

ankle joint is passively relaxed this minimum degree of  $\gamma$ -static innervation due to the effect of gravity will further diminish and the posture thus obtained (which we call AI (1, 4)) will be proportionately reduced. In a normal individual the ratio AI/AR indicates the proportion of  $\gamma$ -static versus  $\gamma$ -dynamic innervation whereas the index AI/M will indicate the proportion of  $\gamma$ -static versus the total pool of motoneurons. An increase or decrease in LVST activity will respectively increase or decrease the value of AI/AR and AI/M.

The Jendrassik manoeuvre has been shown to provoke an increase in facilitatory supraspinal influences primarily of  $\gamma$ -dynamic and static motoneurons (J Paillard). AI and R are effectively increased during the manoeuvre in normal subjects. The degree of augmentation is inversely proportional to the degree of excitability of motoneurons. Thus in spastic patients in which the loop is already saturated the effectiveness of the Jendrassik manoeuvre to increase AI—and still more the AR—is reduced or even abolished. Inversely, desaturation of the  $\gamma$  loop by a lack of activity of facilitatory influences will relatively increase the effectiveness.

The excitability of  $\alpha$ -motoneurons is measured by the index H/M and by the recovery curve of the H reflex (J W Maglader). Passive relaxation of the ankle joint or the Jendrassik manoeuvre induces very little if any modification of the value of H and did not modify the duration of the refractory periods of motoneurons at all (N Porlier et al). Increase in  $\alpha$ -motoneuron excitability is mani-

fested by an increase in H/M and a shortened refractory period. Conversely a reduction in the  $\alpha$  motoneuron excitability induces a fall in H/M and a lengthening of the refractory period.

## MATERIAL

In order to determine the function of the LVST in man we compared the values of stretch reflexes in 54 healthy persons with those of patients with hyper and hypofunction of the vestibular system. The former group

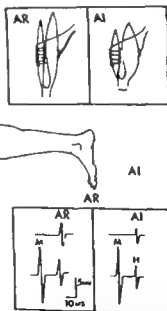


Fig 4 With the gastrocnemius in a completely relaxed position the mechanically induced reflex AI is smaller than the reflex obtained with the foot in a natural resting position called AR. The H reflex and the M response have the same value in both instances.



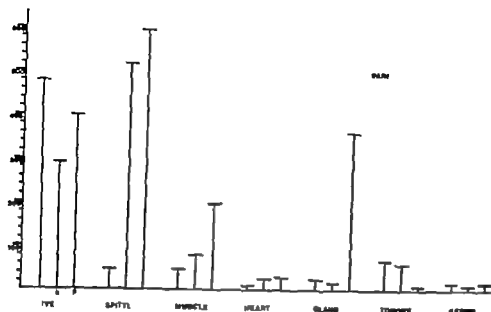


Fig. 3. Variance of the copper values found.  $\mu$  = mean;  $\sigma$  = standard deviation;  $\sigma^2$  = variance.

## RESULTS

In normal animals the values obtained were

|        |                         |
|--------|-------------------------|
| serum  | 9.52 $\gamma/\text{ml}$ |
| saliva | 16.42 $\gamma/\text{g}$ |
| liver  | 56.80 $\gamma/\text{g}$ |
| muscle | 11.84 $\gamma/\text{g}$ |
| heart  | 10.46 $\gamma/\text{g}$ |
| gland  | 10.37 $\gamma/\text{g}$ |
| tongue | 10.46 $\gamma/\text{g}$ |

It is evident that the copper concentration in the liver is much higher than that in the remaining organs and fluids.

In the organs studied and in serum concentrations were similar but a little higher in saliva (Fig. 1). As we can see in Fig. 2 the dispersion index is low and the same is found with the variance values except in the liver (Fig. 3).

All the animals treated with Acetyl homocysteine could not distinguish fresh water from quinine chloride solution between 20 and 30 days giving the same result in the cases in which we used other tastants. We therefore came to the conclusion that taste was eliminated and the animals were killed.

In so far as the D-Penicillamine treated animals are concerned the results were different because we could not obtain ageusia in any case. We lost many animals by increasing the dosage or prolonging the time. It was neces-

sary to include more animals to maintain the required number. We examined these animals on the 25th day of treatment by using a battery of different concentrations in order to detect slight hypogeusias. Only in 17 cases did we obtain it for all four qualities. The rest were excluded. Copper was also investigated and the values obtained were practically the same as in the hypogeusic animals.

In the animals treated with Acetyl homocysteine in which we obtained ageusia, the results were

|        |                          |
|--------|--------------------------|
| serum  | 12.03 $\gamma/\text{ml}$ |
| saliva | 26.96 $\gamma/\text{g}$  |
| liver  | 28.76 $\gamma/\text{g}$  |
| muscle | 13.49 $\gamma/\text{g}$  |
| heart  | 10.54 $\gamma/\text{g}$  |
| gland  | 8.98 $\gamma/\text{g}$   |
| tongue | 9.84 $\gamma/\text{g}$   |

The liver copper concentration fell in relation to normal animals from 56.80 to 28.76  $\gamma/\text{g}$  (Fig. 1) a significant decrease. As we can see in Figs. 2 and 3 there is an evident discrepancy between the figures of variance and dispersion.

In saliva the amount is clearly increased (from 16.42 to 26.96) and slightly so in serum

Table II Modification of ipsilateral stretch reflexes following caloric ( $0^{\circ}$ ) stimulation (1 case)

|                   | H reflex | H/M                 | AI    | AR   | AI/AR | AI+J         | AR+J        | AI/H | AI/M | AR/H | AR/M |
|-------------------|----------|---------------------|-------|------|-------|--------------|-------------|------|------|------|------|
| Normal            | 5        | 31%<br>M. 18<br>mVs |       | 5    | 40%   | 4<br>(+100%) | 8<br>(+60%) | 40%  | 12%  | 100% | 31%  |
| mg<br>stimulation | 6        | 37%                 | 4     | 8    | 50%   | 5<br>(+5%)   | 8<br>(0)    | 66%  | 5%   | 133% | 50%  |
| Excitability      | 20%      | 6%                  | +100% | +60% | +10%  | -75%         | -100%       | +76% | +13% | +33% | +25% |

### Group 2 Temporary vestibular hyperfunction following caloric ( $0^{\circ}$ ) stimulation

In this experiment was performed in a single case of benign positional vertigo. With the patient lying on his back, the stretch reflexes were provoked with mechanical and electrical stimulation. A few seconds after commencing rotation a marked increase in AI and a moderate increase in AR were detected (Table I). The effect was bilateral but more pronounced ipsilaterally. Since the values of AI and AR are increased the effectiveness of the Jendrassik's manoeuvre is proportionally reduced. Caloric activation of the labyrinth could therefore seem to enhance the excitability of  $\gamma$ -static and to a lesser degree  $\gamma$ -dynamic motoneurons.

### Group 3 Modification of stretch reflexes following surgical lesion of the vestibular nerve

Eighteen patients with various vestibular pathologies were selected in whom pre and post-operative studies of myotatic reflexes were performed. The results (Table III) correspond exclusively to ipsilateral reflexes obtained within 7 weeks after surgery. From the clinical standpoint all patients presented a marked abnormal Romberg test that persisted although progressively attenuated for 1 to 18 months. But even after 7 years with the patient's feet placed in front of one another and the eyes closed the Romberg test is still abnormal when the foot on the side of the neurectomy is behind the other.

Immediately after surgery there is a marked fall in the value of AI and a much more moderate decrease in AR and H (Table III). Since Jendrassik's manoeuvre is still strongly positive for AI after surgery it appears that the suppression of vestibular function provokes a selective diminution of the excitability of  $\gamma$ -static motoneurons. Repeated studies after periods of up to 5 years following surgery

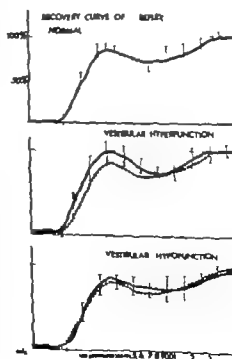


Fig. 5 Recovery curve of the H reflex in normal persons and in patients with hyper and hypofunction of the vestibular system. The dotted lines in the second and third curve correspond to the average normal result.

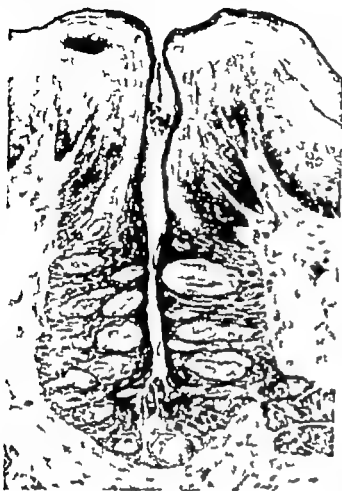


Fig 6 Enlargement of Fig. 5

In serum a slight decrease was obtained (from 9.52 to 8.44) and the same was found in the tongue (from 10.46 to 8.60). As for the rest slight increases were observed (Fig. 1). The dispersion index was increased except in the tongue (Fig. 2) and the variance gave no homogeneous values (Fig. 3).

No macroscopic or microscopic alteration in the tongues / could be detected (Figs. 4, 5 and 6). However in some papillae we could observe a plug of amorphous substance in the entrance to the papillary groove and a lack of amorphous substance in the buds.

## DISCUSSION

It is obvious that Acetyl homocysteine provokes ageusia in guinea pigs but the results are different as far as D-Penicillamine is concerned since we could only obtain slight

hypogeusias in some cases and always below line with toxic effects.

Considering our results as a whole we find that the previous works in which only copper in serum was studied consider the problem only partially as copper metabolism is complex and is carried out mainly in the liver. Hence according to our results in serum we would have to say that D-Penicillamine induces copper whereas Acetyl-homocysteine does not. This was actually the conclusion of previous works. However when we compare the results on the liver we see that it is at a level at which an important decrease takes place in both cases.

In the rest of the organs and fluids studied copper levels were slightly increased. The averages were

Acetyl homocysteine from 11.56  $\mu$ g per ml to 13.63

D-Penicillamine from 11.56  $\mu$ g per g to 13.35

It seems that these drugs deplete copper from the liver at least in the time and at the dosage used. The copper homeostasis is maintained primarily and almost exclusively by biliary excretion (Sass-Kortsak & Beam 1961) and it would have been very interesting to estimate copper concentrations in bile as we did but we overlooked this aspect of the problem. It is possible that copper follows two pathways from the liver: serum and organs and excretion via bile. Thus we may not expect low values in serum until later periods of intoxication.

In the tongue, saliva and salivary glands there is no significant quantity of copper. We think of a direct action of this metal in the taste process. On the other hand from the action of the drugs copper is not decreased as we saw and the result with similar values of metal concentration was different because with one drug we produced ageusia but not with the other. Hence we arrived at the conclusion that copper does not play any part in the taste process. Our figures in the dispersion statistics however are higher in general terms in the animals treated than in the controls. The

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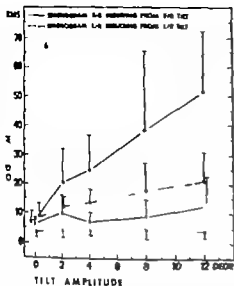


Fig. 4. Mean RMS values (arbitrary units,  $\pm 1\sigma$ ) of lateral and fore-aft stabilograms. Both the lateral and the fore-aft stabilograms were obtained from lateral resp. fore-aft sinusoidal tilt of Tilting Room II (freq. 1/40 Hz, ampl. 2, 4 and 12°). The 6 subjects were standing both without (○) and with (●) foamrubber on top of the stabilometer.

attached to the head and restricting the visual field to 75°. For each condition six stabilograms were recorded, no one or two layers of foamrubber with or without a funnel. It must be emphasized that under these natural conditions it was not possible to control all stimulus parameters of the visual scene or to avoid occasional presence of a slight breeze.

As can be seen in Fig. 3 the instability is already maximal at an eye-object distance of 25 m. No significant difference between the RMS values for the different test conditions obtained at 25, 75 and 200 m could be established. This is also the case when peripheral vision is present. However, when using foamrubber the levels of instability (RMS values) obtained with peripheral vision are below the levels found with the restricted visual field ( $p < 0.01$ ).

The RMS of the stabilograms obtained in the eyes-closed conditions is of the same order of magnitude as the RMS obtained with eye-object distances of at least 25 m without peripheral vision. Ranking the subjects ac-

cording to the RMS in both the balcony and tilting room experiments gives a coefficient of rank correlation of 0.82, indicating that the role of vision in postural stabilization is subject-dependent. Since the postural instability is already maximal at a distance of 25 m the data of experiment I do not sufficiently determine the saturating relationship between eye-object distance and sway amplitudes below 25 m. Therefore a second experiment was designed for testing the influence of small distances as well as the differential effects of optokinetic stimulation on fore-aft and lateral body sway.

#### Experiment II. Lateral and fore-aft body sway at various distances (0.5 to 25 m) and proprioceptive interference

Six healthy subjects (4 male, 2 female, age 20–47) participated in this experiment.

First they were tested in Tilting Room II both with and without foamrubber. The results are shown in Fig. 4. There is a remarkable discrepancy with the results of Expt I. Although the RMS obtained with one layer of foamrubber in Tilting Room II is less than that found in Expt I, the subjects could not perform the test on double foamrubber. Most of the subjects preferred to have some assistance available even with only one layer of foamrubber because they felt unstable and sometimes experienced vertigo.

It is interesting to note that the subjects were much more sensitive to the fore-aft than to the lateral tilt.

The Tilting Room II experiment was followed by a more natural stimulus situation for testing the influence of the distance below 25 m under static conditions. In this experiment a university lecture hall was used to provide different eye-object distances thus eliminating the possibility of wind influencing the results. By a suitable choice of illumination and the use of an adapted funnel the eye-object distance could be varied up to 25 m. The distances in the lecture hall at which stabilograms were recorded were 0.5, 5, 5

Table I Pituitary tumours—clinical and experimental material

|                       | Total | GH | PRL | Chromo-<br>phobe | ACTH | Normal |
|-----------------------|-------|----|-----|------------------|------|--------|
| No. of operated cases | 57    | 31 | 1   | 11               |      | 1      |
| In vitro              |       |    |     |                  |      |        |
| A                     | 31    | 19 | 6   | 3                |      | 1      |
| B                     | 6     | 1  | 3   |                  |      |        |
| Total                 | 37    | 20 | 9   | 5                |      | 1      |

Cases operated on at the ENT Department

\* Cases operated on at the Department of Neurosurgery

turcica. The material is summarized in Tables I and II.

### Methods

#### 1 General considerations

The principles for organ culture of pituitary tumour tissue have been described in detail by Anniko et al (1979a).

Between 24 and 36 pieces from individual tumours were explanted to the in vitro system and observed over a 6–30-day period of culture. The culture media were analysed for both GH and PRL. The nutrient solution was renewed every second day.

All concentrations in the diagrams are calculated as hormone secretion during 24 hours and expressed as percentage of basal secretion i.e. the hormone secretion during the first 2 days in culture.

#### 2 Incubation with bromocriptine

The stock solution of bromocriptine had a concentration of 1:100 000 ( $10^{-5}$  g/l,  $10^{-6}$  µg/ml) dissolved in isotonic saline solution. This was

diluted with culture medium to reach the concentrations  $10^{-6}$ ,  $10^{-7}$ ,  $10^{-8}$  and  $10^{-9}$  g/l, 1:0.1:0.01 and 0.001 µg/ml. Incubation with bromocriptine was performed 2–5×48 h at an initial period of 2×48 h in organ culture medium only.

#### 3 Morphological processing

After ending the culture period in vitro specimens were prepared for light and electron microscopy (cf. Anniko et al 1979a).

## RESULTS

#### 1 The normal pituitary gland

A discrepancy was evident concerning the in vitro synthesis/secretion of GH and PRL respectively (Fig. 1). In all but three organ cultures, totally 24, the hormone concentrations of PRL decreased after a few days in vitro, remaining three specimens showed a reduced synthesis/secretion of PRL from the 6th day on. The GH level in the culture medium increased more rapidly than that of PRL.

Table II Pituitary tumours—type of tumour/lage of the patient

| Type of tumour | No. of patients/age (years) at operation |       |       |       |       |       |     |
|----------------|--|-------|-------|-------|-------|-------|-----|
|                | <20                                      | 20–30 | 30–40 | 40–50 | 50–60 | 60–70 | ≥70 |
| GH             | 1  | 3     | 1     | 4     | 8     | 3     |     |
| PRL            |  | 7     | 4     | 1     |       | 1     |     |
| ACTH           |  |       |       | 1     | 4     |       | 1   |
| Chromophobe    |  |       |       | 4     |       |       | 1   |
| Total          | 1  | 10    | 16    | 10    | 1     | 8     | 1   |

This patient was 13 years old

## SAC DECOMPRESSION FOR REFRACTORY LUETIC VERTIGO

Michael M. Paparella, Chong Sun Kim and Donald A. Shea

*From the Department of Otolaryngology, University of Minnesota, St. Minneapolis, USA*

(Received August 16, 1978)

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Eight endolymphatic sac decompression procedures were done on 6 patients with refractory luetic vertigo in an attempt to relieve their recurrent vertiginous attacks which were intermittent and unresponsive to conservative treatment. We also hope to prevent further hearing impairment.

It is our intention here to describe this problem and to recommend consideration of surgical decompression of the endolymphatic sac in patients with luetic vertigo where extensive medical management has hitherto failed.

## REVIEW OF THE LITERATURE

Luetic hydrops may be seen in both congenital and acquired syphilis. In 1966, Karmody & Schuknecht reported that 38% of 123 congenital syphilitics had a hearing loss, most of whom had associated vestibular symptoms with episodic vertigo and diminished caloric responses. They demonstrated a case of endolymphatic hydrops due to syphilitic osteitis of the otic capsule. Similar findings were observed by Alexander (1928) and Mayer & Frazer (1936). Perlman & Leek (1957) stated that endolymphatic hydrops secondary to invasion of the labyrinthine capsule may have some resemblance to the changes reported in Meniere's disease. This may account for the occasional similarity of symptoms. Schuknecht (1974) suggested that the inner ear reaction to lues is characterized by progressive endolymphatic hydrops and degeneration of the membranous labyrinth resulting in rupture of the membranes due to overaccumulation of endolymph.

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This research has been supported in part by USPHS 1 P01-NS-12125

Table IV *Ratio GH/PRL in GH producing human pituitary tumours in vitro*

Table illustrating three main groups of GH/PRL ratios. Pituitary no. 17 (specimens 19 and 20) has an extremely high secretion of GH approximately 700 000 pmol/l/48 h (cf. Table III). In contrast tumour no. 29 has a synthesis/secretion of GH into the culture medium (2750 pmol/l/48 h). Both tumours have an approximately equal secretion of PRL but the GH/PRL ratios appear completely different. In this case the difference in the GH/PRL ratio indicates differences in the synthesis/secretion of GH. However, tumour no. 20 has a concomitant hypersecretion of GH and PRL. Both hormones are found in very high concentrations in vitro but the GH/PRL ratio is similar to that of tumour no. 29. In conclusion the GH/PRL ratio does not always present the secretory activity of the tumours.

GH pmol/l PRL µg/l

| Specimen no | Days in vitro |       |       |       |      |
|-------------|---------------|-------|-------|-------|------|
|             | 0-            | 3-4   | 5-6   | 7-8   | 9-10 |
| 17          | 19            | 3 469 | 7 710 | 1 113 | 430  |
|             | 20            | 1 667 | 1 813 | 1 153 | 336  |
| 29          | 15            | 69    | 58    | 9     | 5    |
|             | 17            | 6     | 7     | 3     | 4    |
| 0-          | 18            | 3     | 7     | 7     | 9    |
|             | 23            | 9     | 9     | 6     | 4    |

GH cum PRL prior to surgery treated with bromocriptine 3 months

It has to be noted that the in vitro behaviour of the tumour specimens could not be correlated to the tumour size or invasiveness of the sella turcica. Histological sections of more than 400 specimens did not reveal any pituitary cells in mitosis.

### III PRL hypersecreting tumours

In general the PRL hypersecreting tumours adapted better than GH producing tumours to the in vitro system with regard to the synthesis/secretion of hormone. A total of 10 tumours were explanted to in vitro culture.

Table V *GH tumours GH and PRL production in vitro = 1 mg u n = 24 hours*

A significant difference occurs in the secretory capacity of GH between individual tumours as illustrated by tumour no. 17 (specimens 9 and 10) and no. 29 (specimens 13 and 15). The former shows a decrease in the synthesis/secretion of GH with increasing time in vitro. The secretion of PRL increases during this time in culture. The tumour with a low synthesis/secretion of GH appears more stable in vitro. The secretion of GH is approximately the same during the first 8 days after explantation whereas the synthesis/secretion of PRL shows some fluctuations. In conclusion a low synthesis/secretory capacity of a tumour tissue is better preserved unchanged in the artificial environment than tumour tissue having a very high synthesis/secretion of hormone.

| Specimen no | Days in vitro |         |         |         |        |
|-------------|---------------|---------|---------|---------|--------|
|             | 0-            | 3-4     | 5-6     | 7-8     | 9-10   |
| H 17        | 9- GH         | 175 000 | 167 500 | 147 400 | 77 500 |
|             |               | 68      | 48      | 158     | 230    |
|             | 10- GH        | 135 000 | 14 000  | 80 000  | 45 000 |
|             |               | 78      | 34      | 103     | 130    |
| H 29        | 13- GH        | 1 200   | 920     | 980     | 1 450  |
|             |               | 107     | 68      | 70      | 170    |
|             | 15- GH        | 1 030   | 1 045   | 1 080   | 990    |
|             |               | 150     | 18      | 319     | 117    |

up to 3 hours in age 25. Her hearing began to deteriorate progressively in both ears starting 5 years ago. It was fluctuating and progressive, worse on the right ear after a flu-like illness. She had syphilis at age 19 and was treated with Bismuth therapy. She had also received a 70-day course of penicillin therapy 10 years ago.

On admission (August, 1973) audiometric examination revealed a bilateral sensorineural hearing loss with an SRT of 60 dB (R) and 70 dB (L). Discrimination scores were 54% (R) and 58% (L) and SISI scores were 100% on both ears at 4 kHz. Caloric response was markedly reduced bilaterally with very slight subjective sensation in the left ear and none in the right ear. VDRI was reactive and the FTA-ABS test was 4+ in blood and VDRI was negative in CSF. Bicillin was given for 4 weeks with a diagnosis of late latent acquired syphilis. Subsequently additional medical therapy failed to control her symptoms and an endolymphatic sac decompression procedure was performed in September 1973 on the right ear and in February 1974 on the left ear.

She is now rehabilitated. Postoperatively she has had a mild sensation of pressure in her ear and slight imbalance. However she has had no further vertiginous attacks and she is very happy being able to drive her car again.

## DISCUSSION

Histopathologically the lesion of congenital syphilis cannot be differentiated from that of the acquired type. It is characterized by progressive hydrops of the endolymphatic system, degeneration and atrophy of labyrinthine structures with rupture of the membranous labyrinth, causing severe incapacitating episodic vertigo accompanied by nausea and vomiting, fluctuating hearing loss and reduced speech discrimination. Tinnitus, loudness intolerance, sensation of fullness in ears, and diplacusis are also present as in Meniere's

disease. Among those symptoms the most disabling symptom in luetic hydrops is refractory vertigo.

Of our 6 patients, 4 cases initially presented with vertiginous episodes and 2 with cochlear symptoms (hearing impairment and tinnitus). However at the time of admission the patients' chief complaint was incapacitating episodic vertigo which could not be controlled by conservative measures such as penicillin, steroids, erythromycin, antiemetics, sedatives or tranquilizers. Three patients were diagnosed as late congenital syphilis with interstitial keratitis and Hutchinsonian teeth (case 2) and the other 3 patients as acquired syphilis.

Intensive specific anti-luetic medical therapy may fail to prevent the onset of luetic vertigo, deafness or to stabilize the disease process. A hearing loss may occur despite adequate previous antibiotic or steroid therapy due to the variable response of the disease. Our patients had received previous anti-luetic treatment without any significant effect in controlling incapacitating vertiginous episodes or progressing hearing impairment.

In adequately treated syphilis, viable spirochetes are occasionally found in eye, CSF, synovial fluid and lymph nodes and in the temporal bones of penicillin-treated congenital syphilitic deafness. Spirochetes have been found in patients adequately treated with heavy metals. Tamari & Itkin (1951) reported that despite administration of 9 million units of penicillin in 15 days, both hearing and vestibular function continued to deteriorate. The effective level of penicillin in vitreous humor, spinal fluid or labyrinthine fluid against the spirochete is still open to question.

The patient who is allergic to penicillin has a serious problem in treatment. South et al (1964) reported a case of a gravid woman treated with 15 g of erythromycin estolate over a 10-day period 2 months prior to delivery. Her syphilitic infant died and the fetal level of erythromycin was only 1/5 to 1/20 of the maternal level. Case 5 was allergic to penicillin and she was treated with erythromycin for

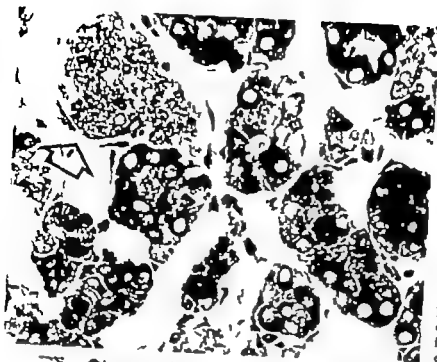


Fig 3 Light microscopy (LM) fixation of prolactinoma tissue (no 19). The tumour is richly vascular. Hormone granules can be observed in cells. One group of cells (arrow) intracellular vesicles (degenerative especially active cells?)  $\times 470$

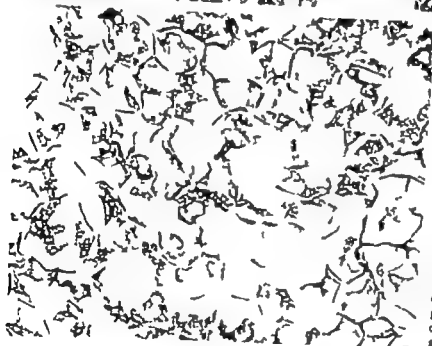


Fig 4 LM Specimen from the same tumour as in Fig. 3. In vitro culture for  $\times 48$  h (basal secretion/adaptation to in vitro culture) but thereafter exposed to 1  $\mu$ g/l (0.1  $\mu$ g/ml) of bromocriptine in culture medium for 4 days. Severe degeneration of cells  $\times 470$

degree to which neural and hormonal factors can affect hormone production (Kohler et al 1969). Also pituitary tumours may be exposed to hypothalamic control factors. As observed in the present study and also earlier reported (cf Tixier Vidal & Farquhar 1975) the synthesis and release of GH and PRL differed with increasing time in vitro.

The maintenance of the secretion of PRL in culture favours a predominant *in vivo* effect of the prolactin inhibiting factor (PIF). On the other hand the decrease in the synthesis/

secretion of GH indicates an *in vivo* effect of a GH-releasing factor. Recently this was also suggested by Peillon et al (1979). However the maintenance of the secretion by pituitary tumour cells in vitro suggests an autonomous secretion although this may be modulated *in vivo* by extra pituitary factors.

Long-term culture of human pituitary tissue has been reported by Lipson et al (1979) describing hormone release but in decreasing concentrations during six months in vitro. Anniko et al (1979b) have reported on pituitary

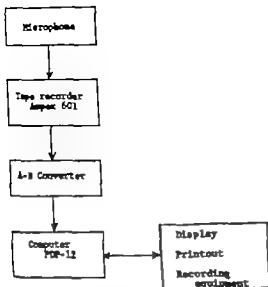


Fig 4 Block diagram of the system

extracted. It should be noted that the third harmonic on the display is actually the first harmonic of the voice waveform (Fig. 3)

## METHOD

A block diagram of the system is shown in Fig. 4

### 1. Voice recording

The subjects were 14 males and 14 females with no laryngeal or pulmonary disorders and 20 males and 10 females with various laryngeal diseases resulting in various degrees of hoarseness (Table 1)

A recording of each subject's voice was

Table 1 Types of voice disorders

| Type of disorder          | N of cases |
|---------------------------|------------|
| Recurrent nerve paralysis | 11 (2)     |
| Atrophy                   | 7 (3)      |
| Polyp                     | 7 (1)      |
| Polypoid degeneration     | 1          |
| Carcinoma                 | 1          |
| Nodule                    | 1          |
| Acute laryngitis          | 1          |
| Metastatic voice disorder | 1 (1)      |

Numbers in the parentheses represent postoperative

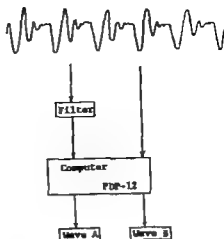


Fig 5 Voice wave digitized without filter (B) and with filter (A)

made in a sound-treated booth using a Electrovoice model 666 microphone and an Ampex model 601 tape recorder. A stable portion of the sustained vowel /a/ was used for the analysis.

The voices on the magnetic tape were edited and arranged randomly in order to avoid listener judgment bias. Five persons who had experience in voice study were asked to judge the degree of hoarseness for each subject. The degree of hoarseness was rated as follows: 0=none, 1=slight, 2=fair, 3=extreme, and the scores were averaged for each subject.

### 2. A/D conversion

A 325 msec section of the voice signal was filtered with a 600 Hz low pass filter and digitalized (wave A). The same section of the voice signal was digitalized without filtering (wave B) (Fig. 5). The sampling rate of the A/D conversion was 22026 points per second. This rate was determined by the sampling program, and both waves were stored on computer data tape.

### 3. Pitch extraction

Wave A was displayed on a scope and three pitch periods ( $T$ ) were measured using a cursor. Fourier analysis was performed on the

cell morphology and hormone secretion has also been reported by Anniko et al (1979c) in prolactinomas exposed to bromocriptine *in vitro* though they found that morphological damage seldom takes place.

Contradictory findings have thus been reported in the literature. One explanation might be the fact that the tumour biology varies considerably among individual tumours although hypersecreting the same type of hormone thereby also giving different results following pharmacological or surgical treatment.

### ZUSAMMENFASSUNG

Von 56 operierten Tumoren hatten 31 eine Übersekretion an Wachstumshormon (growth hormone = GH) 12 Tumore eine vermehrte Sekretion an Prolaktin (PRL) eine Übersekretion an ACTH und 11 Tumore waren chromophob ohne Hormonsekretion. Eine Hypophyse mit erhöhter Hormonausscheidung zeigte normale Verhältnisse bei der chirurgischen Untersuchung was bei einer erweiterten endokrinologischen Kontrolle bestätigt werden konnte. 36 hormonausscheidende Tumore waren einer *in-vitro*-Kultur unterworfen worden. 20 Tumore mit Akromegalie, 9 Prolaktinoma, Bildung von ACTH und 5 chromophobe Adenoma. Je größer der Grad an klinisch nachweisbarer Akromegalie war, desto höher war die Konzentration an Wachstumshormon *in vitro*. Tumore mit einer Übersekretion an Wachstumshormon zeigten auch eine Ausscheidung von kleinen Mengen Laktationshormon *in vitro*. Das Verhältnis GH/PRL war für jeden Tumor individuell, der eine Übersekretion an Wachstumshormon oder Prolaktin hatte. Das Tumorgewebe zeigte ungefähr die gleichen charakteristischen Merkmale *in vitro* unabhängig davon ob das Gewebe verschiedenen Teilen des Tumors entnommen worden war. Eine gute Korrelation zeigte sich bei der Sekretion von Prolaktin *in vivo* und *in vitro*. Je höher der Serumspiegel und Prolaktin was, desto größere Konzentration an Laktationshormon wurde in den *in-vitro*-Kulturen gefunden. Eine Mitose war in keinem der histologischen Schnitte von mehr als 400 verschiedenen Proben nachweisbar. Die Zunahme an Prolaktin in *in vitro*-Kulturen ist wahrscheinlich abhängig von dem Fehlen eines das Prolaktin hemmenden Faktors (Prolactin-inhibiting factor = PIF) bei der *in vitro*-Kultur. Das Absinken des Niveaus an Wachstumshormon dürfte im Gegenteil abhängig sein von dem Fehlen eines das Wachstumshormon freisetzenden Faktors. Die Ergebnisse der vorliegenden Studie deuten auf eine individuelle Biologie in jedem hormonausscheidenden Tumor hin.

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Fig. 12. Sudden change in voice quality (case 9).

I



Fig. 12. Sudden change in voice quality (case 9).



Fig. 13. Sudden change in voice quality (case 13).

tion or vocal shimmer was analysed by Koike (1969), von Leden & Koike (1970) and Kitajima (1976). The previously mentioned investigators using computer analyses, have been successful in evaluating a rough quality which is related to an irregularity of the fundamental cycle.

When combined with the previous methods, the calculation of the S/N ratio of hoarse voice can distinguish a breathy voice from a rough voice. Furthermore the S/N ratio of hoarse voice obtained by the present method appears to be a quantitative indicator of the degree of hoarseness thereby providing objective information on the effectiveness of individual treatment.

## ZUSAMMENFASSUNG

Die harmonischen Bestandteile in der menschlichen Stimme werden von dem Lärminhalt durch einen kleinen Labordaten-Computer abgetrennt. Das Verhältnis von Harmonie zu Lärm oder H/L-Verhältnis (S/N Ratio) wird berechnet und verglichen zu dem Gehörseindruck von 51 Personen. Die kultivierten Resultate zeigen einen Zusammenhang zum Gehörseindruck und es wurde vorge-

schlagen, daß dieses eine hilfreiche Methode sein würde für eine quantitative Bewertung von Heiserkeit.

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## BIOMECHANICS OF THE GIRAFFE LARYNX AND TRACHEA

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**Abstract** Despite possession of a well developed larynx and a gregarious nature the Giraffe is able to utter only low moans or bleats. Morphological and histological examination together with measurements of trachea and subglottic area on three fresh larynges (*Giraffa camelopardalis*) has made it possible to explain the lack of vocal power. Factors such as thoracic expiratory flow rate, length of trachea and recurrent laryngeal nerves together with morphological details of vocal folds and intrinsic laryngeal muscles have all been considered providing a unique example of the relationship between morphology and function in the mammalian larynx.

Although the suborder Ruminantia contains many cud-chewing artiodactyles, probably the most fascinating family are the Giraffidae. This family is only found in Africa and includes two genera—the Giraffe and the Okapi. It is accepted that giraffes are the tallest living animals and their strange appearance caused consternation when first seen in Caesar's Rome around 50 B.C. The Romans called it *Camelopardalis* for the Greeks thought it to be a mixture between camel and leopard. One thousand years later the Arabs named it *Zarafah* (one who walks quickly) and in Italian *Giraffa*. Its scientific name is a combination of old and new—*Giraffa Camelopardalis*!

Giraffes are browsers feeding on leaves and shoots at the top of acacia and other trees. For this they need long necks and although having only 7 cervical vertebrae can grow to a height of over 4.6 m (15 feet) with an adult weight of almost 2 030 kg (2 tons). The body is relatively short resulting in a neck which can measure almost 2.5 m (8 feet) in length. For the purpose of grading, size is related to height with large males being over 4.6 m, females 4 m and immature animals being less than 2.5 m.

Since a 1.6 m column of blood exerts a pressure nearly equal to 125 mm of mercury, the arterial pressure at heart level of a standing giraffe must exceed this in order to perfuse the brain. Measurements have shown that blood pressure at brain level is 215 mmHg rising to 350/250 mmHg when the head is lowered to drink. Despite the physiological hypertension, arteriosclerosis is rarely found at autopsy and this was substantiated in my own examination of the carotid vessels of three specimens which died in captivity.

Although Giraffes are gregarious animals living in family groups or large herds, relationships are loose and animals often join or leave without apparent reason. Parental bonds between mother and young are weak and since suckling periods are short the immature animal may wander away from parents to join other groups or even perish. Almost all field studies agree that vocal communication seems to be minimal, restricted to an occasional snort or at most a weak bleat or moan. Communication seems to be visual, taking advantage of their great height and large eyes. Sudden panicky movement by one animal may result in a stampede of the whole herd even though no visible danger is apparent. Being shy and inoffensive creatures, giraffes' defence against predators is primarily speed. However, their forefeet are powerful and it has been known for an enraged bull to crush the head of a lioness. A galloping giraffe may appear ungainly and relatively slow. However, top speeds of over 56 km per hour (30 mph) have been recorded and this cannot be matched over distance even by a horse.

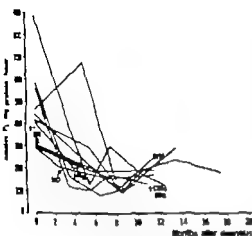


Fig. 1 Eleven patients with initially increased ATPase activity. In 10 cases the activity decreased to normal levels after radiotherapy. Four patients died within the control period and their individual survival time (in months) indicated by figure in brackets. ND Neck dissection. R Remaining tumour mass or relapse. M Metastatic disease.

creased lymphocyte ATPase activities. Individual values are shown in Table I in which the staging of the tumours and the clinical response to treatment in these patients also appear. Following radiation therapy the increased ATPase activities were normalized in all these patients (Fig. 1) except in one namely a patient who died before the first control. The lymphocyte ATPase activity was not normalized also in 7 of the patients despite per-

sisting tumour mass in one and bilateral lymph node metastases in the other.

In 7 patients (Table II) the ATPase activity rose temporarily from an initial normal level to significantly increased values (Fig. 2) after radiotherapy. This observation was not in any case related to the clinical course of the disease. Only 1 of these patients died from relapse and metastases within the control period (29 months after therapy).

The remaining 27 patients (Tables III and IV) also had normal ATPase activities before radiotherapy and the activity remained normal in 19 of these patients who were followed repeatedly after treatment (Fig. 3). In no case did the lymphocyte ATPase activities seem to be correlated to the clinical course of the disease. Three of the patients who had a remaining tumour mass after radiotherapy were operated on and they finally succumbed to their disease (Table III). Two other patients died from causes not related directly to their neoplastic disease.

After a mean observation period of 30 months 36% of the patients had relapsed among those having an initially increased ATPase activity whereas only 15% relapsed among the patients with a normal ATPase activity before therapy. However this difference is not significant ( $p > 0.2$ , Fisher's exact test).

A clinical evaluation of the patients after

Table II Seven patients with initially normal lymphocyte ATPase activity but in whom the activity later increased after therapy (Fig. 2)

At 3-year follow-up after therapy one patient (14%) had died of tumour relapse and metastases. TL Total laryngectomy PL Partial laryngectomy

| Initial | Age (yrs) | Sex | Tumour localization | Tumour stage                                 | Effect of treatment | Operation | Length of observation period (months) | Relapse at the end of observation | Initial ATPase activity (nmol/mg/h) |
|---------|-----------|-----|---------------------|--|---------------------|-----------|---------------------------------------|-----------------------------------|-------------------------------------|
| TAMN    | 63        | ♂   | Glotis              | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Incomplete          | TL        | 42                                    | -                                 | 153                                 |
| EGK     | 81        | ♂   | Supraglot           | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | TL        | 29                                    | +                                 | 153                                 |
| OC      | 46        | ♂   | Glotis              | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Incomplete          | PL        | 38                                    | -                                 | 46                                  |
| GEB     | 77        | ♂   | Glotis              | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            |           | 43                                    | -                                 | 198                                 |
| TTL     | 52        | ♂   | Glotis              | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            |           | 42                                    | -                                 | 86                                  |
| IKB     | 86        | ♂   | Glotis              | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            |           | 44                                    | -                                 | 140                                 |
| AA      | 63        | ♂   | Glotis              | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            |           | 36                                    | -                                 | 263                                 |



Fig 3 Macroscopic view of the commencement of trachea and oesophagus. The right recurrent nerve lies between these two structures and passes between the lowest fibres of the inferior oesophageal muscle. The right paraesophageal muscle is on the lateral surface of the oesophagus. The superior laryngeal muscle is superiorly.

been made to measure the volume of the trachea directly by determining water capacity. Patterson et al. estimated a volume of 2.5 litres in a 16 foot (4.9 m) giraffe whilst Robin et al. (1960) estimated tracheal volume by assuming the trachea to be a cylinder and applying the formula

$$V = \pi r^2 l$$

where  $V$  = tracheal volume in ml,  $r$  is the mean radius in cm and  $l$  is the length in cm. He calculated the volume in one animal as 1.6 litre. In fact the trachea of the giraffe is oval in section (Fig. 1) and in the largest male in my specimens (15 years old) was 51 mm widest diameter, 35 mm shortest diameter with a tracheal length of 2.3 m. This gives an approximate dead space volume of 3.0 litres although this does not include the volume of pharynx, bronchi and bronchioles and is thus an underestimate. Dead space volume in the 13 year female trachea diameter 45 mm  $\times$  28 mm was estimated as 1.8 litres. Only a few giraffe lungs have been weighed and these figures have not always been correlated with the animals size or weight. Figures vary from 6 to

12 kg and in my two adult specimens for fixed lungs weighed 7.2 kg for the male, 6.42 kg for the female. Patterson et al. carried out *in vivo* studies related to lung volume: pulmonary ventilation on three giraffes; their results may not be reliable since the animals were restrained or tranquillised. However it may be supposed that by comparison with man—and the giraffe is at least 6 times heavier—volume of dead space is approximately nine times greater, total lung capacity about eight times larger and tidal volumes range from 2.7 to 4.1 litres. It appears that the giraffe may use two mechanisms for maintenance of alveolar ventilation. The economical way to breathe through a long tube is to do it slowly with large infrequent breaths, expelling dead-space air before it can ventilate the lungs. In conditions of captivity giraffes breathe about 8 to 12 times per minute. In addition, total lung capacity may be unusually large, though the merit of this is to increase inspiratory and expiratory capacity. This is of great importance when the giraffe is running and expiration is probably helped by diminution of the tracheal lumen by contraction of the muscle which is found posteriorly beneath the

Table IV Eight patients with initially normal lymphocyte ATPase activity and in whom only one ATPase determination was carried out

At follow-up 3 years after therapy one patient (125E) had died from tumour relapse and metastases

| Initials | Age (yr) | Sex | Tumour localization | Tumour stage                                 | Effect of treatment | Operation | Length of observation period (months) | Relapse at the end of observation | Initial ATPase activity (nanoles P <sub>i</sub> /mg/h) |
|----------|----------|-----|---------------------|--|---------------------|-----------|---------------------------------------|-----------------------------------|--|
| PP       | 39       | ♂   | Supraglott          | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 25                                    | -                                 | 170  |
| APB      | 61       | ♂   | Glottis             | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Incomplete          | -         | 10                                    | +                                 | 105  |
| EFD      | 59       | ♀   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 26                                    | -                                 | 165  |
| AK       | 54       | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 26                                    | -                                 | 138  |
| LAL      | 66       | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 26                                    | -                                 | 195  |
| SEJ      | 54       | ♂   | Glottis             | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 25                                    | -                                 | 104  |
| FC       | 65       | ♂   | Glottis             | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 28                                    | -                                 | 169  |
| SAL      | 51       | ♂   | Glottis             | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 26                                    | -                                 | 58   |

Tumour cell specific sensitization of the killer cells could be due to circulating tumour cell associated antigens, but non-specific killing may also be effective (Pross & Baines, 1977). The energy needed for the cytotoxic potential details of which are still not understood could be provided by hydrolysis of ATP by mitochondrial Mg-ATPase. As a matter of fact it has previously been shown that a mitochondrial ATPase activity is present in human nor-

mal lymphocytes in the blood (Ellegaard & Dimitrov 1973) and that this ATPase activity is increased in patients with various neoplastic tumours including carcinoma of the larynx (Ellegaard & Dimitrov 1972a 1972b Dimitrov & Ellegaard 1973 Ellegaard et al 1975a 1975b 1975c). Contrary to what was believed from the beginning it seems to be the B-lymphocytes which carry the highest ATPase activity in unstimulated normal human lymphocytes isolated from the blood (Kragballe & Ellegaard 1978) and elevated ATPase activity is also found in lymphocytes from patients with B-lymphocyte proliferative diseases like chronic lymphocytic leukaemia (Ellegaard 1979).

The normal range for the lymphocyte ATPase activity is higher in the present study than in the previous investigation (Ellegaard et al 1975c). This is most likely due to alterations in the methods omitting removal of the sticky populations of cells by passing the mononuclear cell suspensions through glass-bead columns. The present study has confirmed that the ATPase activity of circulating lymphocytes is increased in a number of patients with carcinoma of the larynx and that the ATPase activity in these cases decreases to normal levels after radiotherapy. Increased protein RNA and DNA synthesis has been demonstrated in human lymphocytes transformed

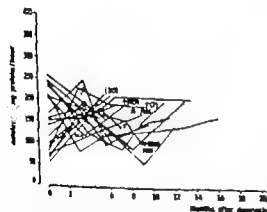


Fig. 1 Nineteen patients with initially normal or sub-normal ATPase activity. After radiotherapy the ATPase activity decreased in 7 patients, while it increased in the 11 other cases. Five patients died within the control period, 3 from relapse or metastases, the 2 from causes not related to their malignant disease. PL Partial laryngectomy T2 Total laryngectomy R Relapse RM Relapse and metastases.



Fig. 5 This is a section taken deeper to that shown in Fig. 4 and shows the large arytenoid and attached fibres of the thyroarytenoid muscle.



Fig. 6 Section through the right half of the larynx of an adult female graffie. This illustrates the large cartilage, Wrisberg bracing the aryepiglottic fold and the broad attachment of the thyro-arytenoid muscle to the front of the arytenoid.

geal nerves of 3 human larynges. He counted the total number of myelinated fibres in both the internal and recurrent laryngeal nerves finding a total of approximately 4000 with a unimodal distribution, the peak lying between 8–12  $\mu\text{m}$ . Suggestion that these nerves are a plexus of the vagus represented by a continuous nerve joining internal and recurrent nerves and that separation from this strand forms the various laryngeal nerves" would certainly explain the numerous variants found in the mammalian larynx.

Since the electrophysiological and biochemical parameters of nerve fibre function are related to fibre diameter and the longer myelinated alpha motor fibres lie in the Group I range (up to 25  $\mu\text{m}$ ) the number of these fibres in the recurrent nerve are of some im-

portance when considering laryngeal function. In the human fibres of 10  $\mu\text{m}$  in diameter would allow a conduction velocity of about 50–65 m  $\text{S}^{-1}$  with longer refractory period than in motor nerves to the limbs. In view of the considerable length of the recurrent laryngeal nerves in the graffie they commence within the thorax as in man, it is of interest to examine the number and size of the large fast conducting myelinated fibres within this nerve. The technique used is based upon the described by Holland (1978) the specimen being stained with uranyl acetate and embedded in Epon. Details of these findings will be described later when all six nerves have been studied for accurate measurement is tedious and time consuming. Myelinated nerves pre-

chiae and ecchymoses which tend to bleed easily. Localized deposits of amyloid are common in oral plaques, diffuse thickenings or translucent papillae. Amyloid infiltration has been reported in certain odontogenic tumors (Vickers et al 1965). The deposition of amyloid frequently precedes the calcification in the tumor.

There have been only a few reports of amyloid deposits in the tonsils (Mutachler 1933; Charr 1937; Eriksen 1970). In these, the presenting symptoms varied from no apparent disturbance to severe obstruction.

The present study describes a unique case of amyloidosis of Waldeyer's ring and electron microscopic observations of large amyloid deposits and the cells surrounding them in palatine tonsils.

## CASE REPORT

A 35-year-old male patient, born in Morocco, was admitted to our department in February 1977. Six years prior to the present admission he was treated for acute maxillary sinusitis. Three months before admission, he had an attack of acute tonsillitis, which was treated successfully by ampicillin. Five weeks later another episode of tonsillar infection occurred. He was referred to our outpatient clinic because of a mild feeling of discomfort in the throat which had persisted since the last episode of infection.

On examination, both tonsils were found to be enlarged and firm. Several yellowish plaques were observed in their anterior surface and an additional large plaque, measuring 3.0 × 4.0 cm, was seen over the left posterior pillar. Several yellowish masses occupied the base of the tongue and encroached on the epiglottis. Another mass was seen in the left vallecula. A plaque, 0.3 cm in size, spread upward from the upper border of the oropharynx to the lateral wall of the nasopharynx on the left side and another small plaque was seen in the middle of the posterior wall of the

nasopharynx. The patient was in good physical condition and examination revealed no other pathological findings.

Laboratory findings: peripheral blood hemoglobin 13.8–15.0 g/dl, ESR (Westergren) 10 mm in the first hour, leukocytes 5700–7700 per mm<sup>3</sup> with a normal differential count. Urine analysis, including microscopic examination of sediment, disclosed no abnormality. Blood levels of glucose, urea, uric acid, albumin, globulin, creatinine, bilirubin, cholesterol, electrolytes, alkaline phosphatase, SGOT, transaminase, prothrombin, calcium and phosphorus were all within the normal range. Creatinine clearance was 125 ml/min, serum folate 8.8 ng/ml, serum B<sub>12</sub> 266 pg/ml. Serologic tests: Wassermann, Kahn, VDRL, cold agglutinins, Rose-Waaler, Paul-Bunnell latex, rheumatoid factor, antinuclear factor, monostest were all negative. Anti-DNA level was 12.1%. LE cells were not found. Measurement of blood immunoglobulins showed IgA 168 mg%, IgM 67 mg% and IgG 1830 mg%.

In vitro stimulation of peripheral blood lymphocytes by lectin mitogens (phytohemagglutinin, Concanavalin-A, pokeweed mitogen) was normal.

Tonsillectomy and biopsy of the nasopharyngeal and hypopharyngeal tissue masses were done. All the studies revealed amyloidosis as determined by Congo Red staining. Biopsies of rectal and buccal mucosae did not show any signs of amyloid deposits.

## METHODS

Different regions from the excised right tonsil were fixed in 2.5% glutaraldehyde in cacodylate buffer, pH 7.4, for 2 hours and post-fixed in 1% osmium tetroxide for one hour. Following dehydration in a graded ethanol series and propylene oxide, they were embedded in Epon 81 (Luft 1961). Semithin sections were stained with toluidine blue, ultrathin sections were stained with uranyl acetate and lead citrate.

Mitwirkende Umstände wie expiratorische Ausflusgeschwindigkeit Länge der Trachea und des Nervus recurrens sowie morphologische Details der Stimmfalten und intrinsischen laryngealen Muskeln wurden alle in Betracht gezogen. Sie gaben uns ein bemerkenswertes Beispiel der Beziehung zwischen Morphologie und Funktion der saugtier Larynx.

## RESUMÉ

Bien que la girafe (*Giraffa camelopardalis*) soit munie d'un larynx largement développé et d'une nature grégaire elle n'est capable que d'émettre des éléments et des gémissiments dans le registre grave. L'examen morphologique et histologique accompagné de mesures de la trachée-artère et de la zone subglottique de trois larynx fraîchement prélevés a rendu possible une explication de ce déficit de force vocale. Ces facteurs suivants ont été considérés: la vitesse d'écoulement de l'air expiré du thorax; longueur de la trachée-artère; longueur de la branche récurrente du nerf pneumogastrique; les détails morphologiques des cordes vocales et la structure des muscles intrinsèques du larynx. Ces facteurs offrent un exemple unique de la relation entre la morphologie et la physiologie du larynx chez les Mammifères.

## ACKNOWLEDGEMENTS

I wish to express my thanks to Marwell Zoological Gardens for the larynx and trachea of VICTOR and to the Zoological Society of London for the two specimens of Giraffe larynges with trachea.

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## DISCUSSION

*Diamond to Harrison:* What happens to race-horses if have the condition called 'roaring'? Their efforts suddenly diminish and they slow down while running. What is the pathogenesis?

*Townsend to Harrison:* Did I understand you correct when you said the lack of tone pitch is due to the low speed? The pitch is solely determined by the tension of the vocal cords if you disregard the rare case of overblown when the pitch goes up one octave.

I would suggest that the air flow is too low to set the vocal cords vibrating.

*Sadé to Harrison:* Can the okapi the giraffe's close speak? It seems to have a neck no larger than the lion's.

*Spoendlin to Harrison:* I wonder how the great coughs, a function of the larynx which presumably important in an animal with such a long trachea.

### Harrison (Reply)

Roaring in the horse is due to left recurrent nerve palsy and is found in the large draft animals or the genetically related inbred racehorse. Pathological studies suggest that this is probably secondary to dilatation of the left aortic Orator's Syndrome. Surgery is unsuccessful as the animal requires a large glottis for effective activity such running.

High subglottic pressure is only one of the necessary factors for good sound production. However the large dead space in the giraffe makes flow rates unusually low. Undoubtedly the long recurrent nerve and possibly the conduction time together with simple type of larynx add to this animal's problems.

Cough is not a feature of the giraffe's behaviour probably because of the high protective aryepiglottic folds in herbivorous diet.

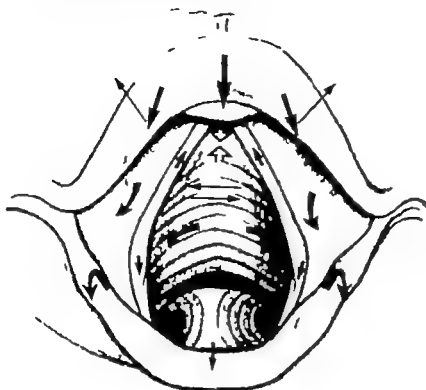


*Fig 7* Vacuoles containing varying amounts of vesicles, granular material, small dense bodies, and empty regions. Note numerous profiles of RER (arrowhead) and small vesicles (arrow) in the cytoplasm. *M*, mitochondria. 13000

*Fig 8* Clear vacuoles, some of which contain membranous and myelin-like components (arrow). *N*, nucleus. 12180

*Fig 9* Region of vacuolated cell containing numerous mitochondria (*M*) and microfilaments (arrow). *I*, amyloid inclusions. 33060





*Fig 1* Lymphatic drainage of the inner larynx—anterior aspect.

## RESULTS

### *Endoscopic lymphangioscopy*

The lymphatic system of the larynx is composed of two interconnected lymphatic networks i.e. a superficial capillary and a deeper collecting channel system. The density is greatest in the area of the false cord and lowest in the posterior vocal cord. Injection of patent blue into the various areas yields following results:

(a) Injecting the laryngeal aspect of the epiglottis dye is drained to the free margin into the ary-epiglottic fold, to the false cords, to the anterior commissure and penetrating the epiglottic cartilage into the base of the tongue.

(b) From the false cords lymphatic drainage occurs towards the ary-epiglottic folds into the piriform sinus and subsequently following the course of the superior laryngeal artery. Dorsally there is connection to the posterior commissure, the arytenoid processes and the posterior parts of the true vocal cords.

(c) The true vocal cord lymphatics are most ly parallel orientated with some branching towards the ventricle. At the free margin there

is a sharp demarcation towards the subglottic space except for the anterior commissure where a midline crossover is possible underneath.

(d) Injection of the ventricle leads to a quick drainage of dye dorsally via the arytenoid process into the false cords and anteriorly into the anterior commissure. The true vocal cords and the anterior part of the false cord remain unstained.

(e) In the subglottic space the lymphatics run in a circular fashion. A midline crossover occurs as a rule.

It has to be emphasized that during in vivo dye injections active transport of the patent blue leads to the demonstration of a primary lymphatic network and of a collecting channel system far apart from the dye depot. In post mortem specimens the lymphatics are demonstrated at the periphery of the depot and the spread of the dye is—compared to the in vivo studies—limited. The interstitial space is stained in a diffused manner leading to a compartmentation which is not found during in vivo studies.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the advice and assistance of Prof. M. Pras, Sheba Medical Center and thank M. O. Pollack and Mrs G. Buckler of the Photography Department, Municipal Governmental Medical Center, Tel-Aviv. This work was supported by the Research Committee of the Municipal Governmental Medical Center, Tel-Aviv.

## ZUSAMMENFASSUNG

Amyloidose der Mandel ist ein seltener Zustand und über Amyloidose des Waldeyer's Rings wurde bisher nicht berichtet. In der vorliegenden Studie wird ein Fall von Amyloidose der Mandel, des Nasopharynx und der Zungengewebe bei einem 35-jährigen gesunden Patienten ohne klinische Symptome beschrieben. Die Möglichkeit einer systemischen Amyloidose wurde durch klinische Untersuchung und Biopsie von retinalen und buccalen Mucosae ausgeschlossen. Elektronenmikroskopie zeigte das Vorhandensein typischer amyloider Fibrillen. Große Mengen der Fibrillen waren von Zellen, die phagozytisch ersahen aus, nicht eingeschlossen. Die Ultrastruktur dieser Zellen ist beschrieben. Bei diesem Fall handelt es sich um einen eingesparten Typ organbeschränkter Amyloidose des Waldeyer's Rings.

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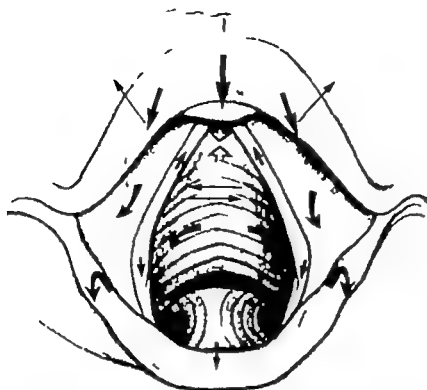
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*Fig 1* Lymphatic drainage of the inner larynx, anterior aspect.

## RESULTS

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(c) The *true vocal cord* lymphatics are mostly parallel orientated with some branching towards the ventricle. At the free margin there

is a sharp demarcation towards the subglottic space except for the anterior commissure where a midline crossover is possible underneath.

(d) Injection of the *ventricle* leads to a quick drainage of dye dorsally via the arytenoid process into the false cords and anteriorly into the anterior commissure. The true vocal cords and the anterior part of the false cord remain unstained.

(e) In the *subglottic space* the lymphatics run in a circular fashion. A midline crossover occurs as a rule.

It has to be emphasized that during *in vivo* dye injections, active transport of the patent blue leads to the demonstration of a primary lymphatic network and of a collecting channel system far apart from the dye depot. In post mortem specimens the lymphatics are demonstrated at the periphery of the depot and the spread of the dye is—compared to the *in vivo* studies—limited. The interstitial space is stained in a diffused manner leading to a compartmentation which is not found during *in vivo* studies.

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*Fig 3* Lymph-collecting vessel of the falls cord with valve *HI* Histiocyte-*LC* vein cell. Inset: Comparison of lymphatic-capillary endothelium (above) and arterial endothelium (below). *Lu* lumen *LC* Lymph capillary *BC* blood capillary *VC* vein cell

vessel level show a sharp and segmental delineation in a horizontal and vertical plane is unfeasible. For the laryngeal lymphatic capillaries midline-crossing anastomoses are seen in the supra- and infraglottis as well as connections with the trachea. It is at the free margin of the true vocal cords where the only sharp demarcation between the capillary system of the supra- and infraglottis can be detected. At

the collecting vessel level there are connections between supra- and infraglottis between epiglottis and the base of the tongue between false cords and piriforme sinus and between infraglottis and trachea. This means—in accordance with clinical observations—that there are multiple superficial and deep interconnections of the laryngeal lymphatic system. The lymphatic drainage always occurs

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*Fig 3* Lymph-collecting vessel of the falls cord with valve *H* Histiocyte *VC* vessel cell. Inset: Comparison of lymphatic-capillary endothelium (above) and vascular endothelium (below) *L* lumen *LC* Lymph capillary *BC* blood capillary *VC* vessel cell

vessel level show a sharp and segmental delineation in a horizontal and vertical plane is unfeasible. For the laryngeal lymphatic capillaries midline-crossing anastomoses are seen in the supra- and infraglottis as well as connections with the trachea. It is at the free margin of the true vocal cords where the only sharp demarcation between the capillary system of the supra- and infraglottis can be detected. At

the collecting vessel level there are connections between supra- and infraglottis, between epiglottis and the base of the tongue, between false cords and piriforme sinus and between infraglottis and trachea. This means—in accordance with clinical observations—that there are multiple superficial and deep interconnections of the laryngeal lymphatic system. The lymphatic drainage always occurs

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- Melnikov O F See Gjulling E V and Melnikov O F
- Messer G See Berser M Messer G Samuel J Gross B and Shanon F
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- Nemančić D See Krmpotić Nemančić J Kostović I Kelović Z and Nemančić D
- Nilsson I M See Harris S and Nilsson I M



take into account not only the larynx damage. For that reason it is very important to know the histological findings of the biopsy extempore in order to know if you are or are not on the right place when making the excision. In the last five years combined partial laryngectomy have given us better results than the classical vertical or horizontal excision.

The second point is that the superficial supraglottic tumor rarely goes to the glottis because unless lymph drainage exists, the linea superior is the barrier for spreading of the tumour. Deep infiltration of the supraglottic space proceeds very rapidly paraglottically in the second or third compartment of the larynx.

*Toppo-ada to Beck.* On the basis of this lymphatic drainage we should limit the indications for supraglottic

and hemilaryngectomy to very early tumours and reserve total laryngectomy for the remaining cases.

*Kleinsasser to Beck.* We should avoid one failure of thinking: a tumor sometimes invades lymph vessels—but the lymphatics are not the main routes of tumor spread. Therefore the decision to perform a partial resection should not depend on the local pattern of the lymphatics because this pattern usually does not correspond to the extension and direction of the local tumor infiltration.

*Beck (Reply)*

*to Naumann Jakob Toppozoda Kleinsasser.* Actually I am not against the partial laryngectomy but due to the results of our investigations we have decided to act in very strict accordance with our indications.

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- Wersäll J See Anniko M and Wersäll J
- Vertes D See Hornstrand C Axelsson A and Vertes D
- Wit H P See Bleeker J D Wit H P and Segenhout J H
- Wright C H See Johnsson L G Wright C H Preston R E and Henry P J
- Ylikoski J See Collan Y Ylikoski J Palva T and Selamaa R
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### Conferences and Meetings

1980 July 9-16 Tenth International Congress on Acoustics to be held in Sydney  
*Australia.* Information 10th ICA Congress Secretariat, GPO Box 2609 Sydney  
 NSW Australia 2001

1980, July 20-23 International Symposium on "Neuronal Mechanisms of Hearing"  
 to be held in Prague, Czechoslovakia. Information, J. Syka, Institute of Experimental  
 Medicine, Czechoslovak Academy of Sciences, 128 08 Prague 2, U nemocnice 2,  
 Czechoslovakia.

1980 September 2-5 IV International Symposium on Facial Nerve Surgery to be  
 held in Los Angeles, California, USA. Further information address Facial Nerve  
 Symposium, c/o Ear Research Institute, 256 South Lake Street, Los Angeles, Cali-  
 fornia 90057 USA.

1980, September 21-22 The XXXI Congress of the Polish Otolaryngological  
 Society will be held in Poznań. Further information. As Professor Antoni Prusze-  
 wicz, Clinic of Otolaryngology Academy of Medicine, 49 Przybyszewskiego Str.,  
 50 355 Poznań, Poland.

1980, September 27-28 The Research Forum, under the joint sponsorship of the  
 Committee for Research in Otolaryngology of the American Academy of Oto-  
 laryngology and the Association for Research in Otolaryngology will be held in  
 Anaheim, California. Abstract format instructions from Professor Makoto Igarashi,  
 M.D., Department of Otorhinolaryngology and Communicative Sciences, Baylor  
 College of Medicine, Houston, Texas 77030, USA.

1980 October The Ear Research Institute announces a two-week Temporal Bone  
 Surgical Dissection Course. Information. Antonio De La Cruz, M.D., Director  
 Temporal Bone Surgical Dissection Course, Ear Research Institute, 256 South Lake  
 Street, Los Angeles, CA 90057 USA.

1980 October 3 A Meeting of the O.R.S. (Oto-Rhino-Laryngological Research  
 Society) will be held at the Royal National Throat, Nose and Ear Hospital, Gray's  
 Inn Road, London. Information Professor P. Stell, Ch.M., F.R.C.S., Department of  
 Otolaryngology Royal Liverpool Hospital, Prescott Street, Liverpool. L7 8XP  
 England.

1981 March 22-27 Second International Conference on Cholesteatoma a  
 Mastoid Surgery to be held in Tel Aviv Israel. Information Professor J. Sadé,  
 International Conference on Cholesteatoma and Mastoid  
 Tel-Aviv Israel.

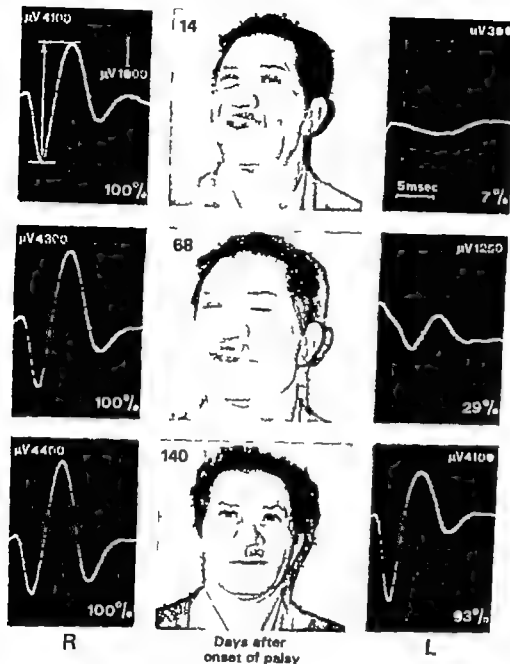


Fig 1 Electroneurogram of a patient with Bell's palsy

tion block since only the former are meaningful with regard to prognosis and hence in the choice of treatment

#### MATERIAL AND METHOD

The examination is performed with a Mk III Amplaid an apparatus commonly employed in the study of evoked acoustic potentials plus a stimulator with its accompanying system of stimulus and recording electrodes

The stimulus consists of a 0.2 msec 50 to 120 V rectangular pulse. Stimulation starts

with near zero voltage pulses at a frequency of 1 per sec and progresses to the point of maximum stimulus i.e. when no further increase in response can be detected on the oscilloscope

The stimulation must be able to activate all the nerve fibres and an intensity of 5-10% more than the maximum is therefore employed. This value depends on certain patient variables such as age and the development of the muscle masses. It will obviously be retained in all subsequent sessions

The surface bipolar stimulation electrode is

of urgency. Rapid, efficient and dependable diagnostic techniques are essential therefore.

Electroneuronography is able to satisfy these requirements in a particularly effective manner. By comparison with other electrodiagnostic methods it has the prime advantage of offering early, exact data that can be readily translated into quantitative values. If the nerve deficiency is more than 90% for example, surgery may be undertaken less than 72 hours after the onset of traumatic paralysis, since the simplicity and rapid execution of the test enables what amounts to a continuous watch to be kept on any progressive degeneration of the nerve.

Evaluation, of course, will depend on the aetiology. However, once it is remembered that a facial defect does not become clinically evident until more than 40% of fibres cease to function, a test capable of detecting changes of only 5% is clearly a valuable guide in assessing both the further progress of a paralysis and the effects of treatment.

False negatives or positives are, of course, inevitable in all forms of diagnostic enquiry. Decisions with respect to treatment, therefore, should definitely be taken in the light of the evidence provided by several examinations which should be repeated in further sessions wherever possible.

## ZUSAMMENFASSUNG

Die Elektoneuronographie (ENoG) besteht in der Aufzeichnung eines durch einen sogenannten elektrischen Reiz hervorgerufenen Summationspotentials der motorischen Endasten. Die Degeneration (und offensichtlich die Unterbrechung) einer Nervenfasern bedeutet immer die Desynchronisation aller sich auf dieselben beziehenden Muskelfasern, so daß der Amplitudenabnahme des Summationspotentials der Anzahl der survivierten motorischen Endasten verhältnismäßig sein wird. Dem Test liegt der Vergleich der Gegenwirkungen, je Reiz gleicher Stärke, zwischen gesunder und kranker Seite zugrunde, was ermöglicht, die Anzahl der Nervfasern des verletzten Teils zuverlässig festzustellen, die gegenüber dem unbeschädigten Teil vermindert sind. Zusatzenutzen der davon ableitbaren Muskelfasern hervorzuheben. Der Verfasser schildert die bei 68 periodisch beobachteten Fällen erhaltenen Ergebnisse und bespricht die Möglichkeiten von diesem einfachen elektrodagnostischen System gebo-

tenen Vorteile bei der Beurteilung der Entwicklung einer Paralyse des Gesichtsnervs und bei der Festlegung genauer und zuverlässiger therapeutischer Angaben vor allem im chirurgischen Gesichtspunkt aus.

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## DISCUSSION

**Stahl to Rossi:** Have you any experience of measuring the area of the entire reaction besides the amplitude?

**Jakobi to Rossi:** Das gute Erfahrungen können wir ausbeutungen, jedoch aber nicht M. Portmann, dass weitere Parameter zur Beurteilung eines Verlaufs de-Facialisparalyse notwendig sind. Der Fingerdruck, mit dem Rossi die Elektroden hebra, lässt können eine Fehlerquelle sein. Wir bevorzugen deshalb für unsere sehr viel kleineren Elektroden den Bestmuskeler.

**Pfaff to Rossi:** A single response in electroneuronography is not very reliable. For this reason averaged responses ought to be used for more reliable evaluation of the global facial response.

**Ehrenberger to Rossi:** Après nos expériences la neuroneuronographie est une méthode éprouvée en cas des paralysies de Bell, mais pas en cas des paralysies traumatiques: même une perte totale de la réaction pendant la première semaine peut être suivie d'une restitution totale de la fonction nerveuse après plusieurs semaines.

**Rossi (Reply):**

Electroneuronography is a very useful test in the diagnosis of facial palsy. Yet it is by no means the only electrical

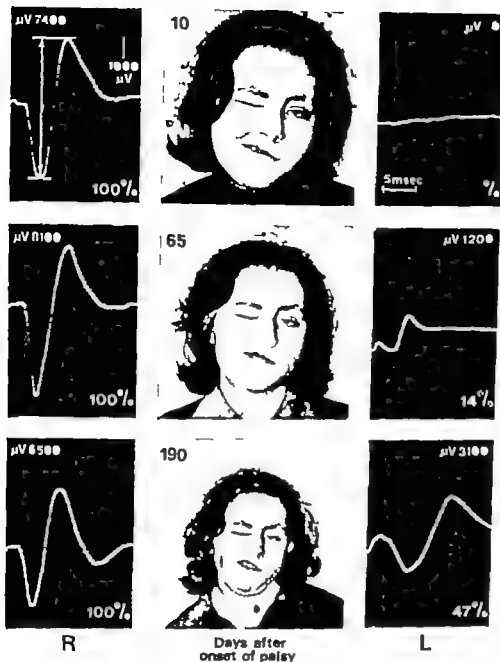


Fig 3 Electroneuronogram of a patient with intratemporal traumatic lesion of the facial nerve and with facial nerve graft.

Diagnosis is based on repetition of the test at intervals determined by the situation and on evaluation of the increase or decrease in response observed. Variations of as little as 5% between one examination and another can be detected.

### CASE MATERIAL

Our series consist of 60 cases: 28 Bell's palsy, 10 Ramsay Hunt palsy, 6 postparotidectomy paralysis, 12 post-traumatic paralysis, 2 post

traumatic paralysis with nerve graft in the intramastoid tract and 2 essential spasm (Figs 1, 2, 3).

### DISCUSSION AND CONCLUSIONS

Electroneuronography is a useful means for monitoring the progress of paralysis. Its most valuable contribution, however, is to the early diagnosis of diseases of the facial nerve.

Treatment of facial paralysis, particularly surgery, must in fact be regarded as a matter

of urgency. Rapid, efficient and dependable diagnostic techniques are essential therefore.

Electroneuronography is able to satisfy these requirements in a particularly effective manner. By comparison with other electrodiagnostic methods it has the prime advantage of offering early exact data that can be readily translated into quantitative values. If the nerve deficiency is more than 90% for example surgery may be undertaken less than 72 hours after the onset of traumatic paralysis since the simplicity and rapid execution of the test enables what amounts to a continuous watch to be kept on any progressive degeneration of the nerve.

Evaluation of course will depend on the aetiology. However once it is remembered that a facial defect does not become clinically evident until more than 40% of fibres cease to function, a test capable of detecting changes of only 5% is clearly a valuable guide in assessing both the further progress of a paralysis and the effects of treatment.

False negatives or positives are of course inevitable in all forms of diagnostic enquiry. Decisions with respect to treatment therefore should definitely be taken in the light of the evidence provided by several examinations, which should be repeated in further sessions wherever possible.

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Die Electroneuronographie (ENOG) besteht in der Aufzeichnung eines durch einen ungesunden elektrischen Reiz hervorgerufenen Somatosensitivpotentials der motorischen Elektroden. Die Degeneration (und offensichtlich die Unterbrechung) einer Nervenfaser bedeutet immer die Denervierung aller auf derselben beruhenden Muskelzweige, so daß die Aufzeichnungsabnahme des Somatosensitivpotentials der Anzahl der ernervierten motorischen Einheiten verhältnismäßig sehr wird. Diese Test liegt der Vergleich der Gegenreaktionen, je Reiz gleicher Stärke, zwischen gesunder und kranker Seite zugrunde und ermöglicht, die Anzahl der Nervenfasern des verletzten Nerven unmittelbar festzustellen, die gegenüber dem unbeschädigten Teil vermindert, das Zusammenziehen der davon abhängigen Muskelzweige hervorruft. Der Verfasser schildert die bei 60 periodisch beobachteten Fällen erhaltenen Ergebnisse und bespricht die nützlichen von diesem einfachen elektrodagnostischen System geboten

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Electroneuronography is a very useful test in the diagnosis of facial palsy. Yet it is by no means the only electrical



diagnostic test that need be employed. Its results must always be supplemented by those obtained by chronaximetry, evaluation of the rheobase and electromyography.

Desynchronization of the response is often noted when the patient is examined more than 30 days after the onset of paralysis. The difference sometimes noted between the amplitude of the response and the extent of functional recovery is probably attributable to such desynchronization.

Like all previous authors in this field, we evaluate the amplitude of the potential only. Latency and the area of the wave are of scant diagnostic or prognostic interest.

We always soaked our electrodes in saline because they are flat and not of the suction-cup type.

When palsy is due to trauma, functional recovery may be noted a few days after the injury. This is the case when concussion is involved, since the phenomena to which it gives rise can be resolved in a few days if suitably treated, and does not represent an interruption of the nerve fibres. If the fracture site cannot be located radiographically, therefore, it is better to wait 15 days before embarking on surgery. This, in turn, will need to be undertaken after the 10th day when daily repetition of all the various electrodiagnostic tests has led to negative results for at least 4-5 days.

## THE R THRESHOLD OF THE QUICK COMPONENT

*A New Measure in Electronystagmography*

M. Pansini and I. F. Padovan

*Zagreb, Yugoslavia*

Vestibular imbalance affects the eyes through the fasciculus longitudinalis medialis and oculomotor nuclei. As regards vestibular excitation, eyes could theoretically be turned around and around until balance is achieved. However, since physical and anatomical limitations do not allow such turning around the eyes could be held in the utmost lateral position in the position of conjugated deviation. Nevertheless, the eyes are not held in this position of conjugated deviation since the compensatory central component brings the eyes back to the position of azimuth zero. Logically, this should depend on physical limitations and on the tension of the eye muscles which transmit information to the central structures via the proprioceptive afferent paths. The speed of the slow component and other elements also plays a certain role here. It seems that we are faced with a great task of determining all these elements in order to define the point where the returning quick eye movement begins.

There are many elements which send commands to revert eyes. Sometimes the eye reverts after a high amplitude and sometimes after a low amplitude. It is not the same in all individuals (Collard & Eber 1977). Many central mechanisms which are involved are not yet fully understood. The number of the unknown elements is too large to allow any possibility of their definition and measurement. (Fig. 1)

However, the first step was carried out by determining the point where the slow component of nystagmus stops and the quick component begins as the point where the mechanism for reverting the eye starts to work. This point

could be called the threshold of the quick component. This means that the point is identified but not explained. Its identification makes it a possible subject for investigation.

*Identification and Measurement of R*

The assumption that this point can be defined only by knowing all the elements which determine it, is quite discouraging. It is possible however to make a comparison between the many elements of nystagmus which are measurable even though their mechanisms are still not well known. We may say that it is not absolutely necessary to know all the mechanisms in order to determine and measure this threshold. On the contrary, by making the threshold of the quick component measurable it serves for purposes of investigation and learning about the elements which determine it.

The next step in our exploration was made when we assumed that if there is a certain threshold of sensitivity for the appearance of the quick component, then it must be constant in the examined individual, no matter whether nystagmus is weak or strong, whether it is at the beginning, in the middle or at the end of the provoked nystagmus. This is called the law of preservation. The Nobel Prize winner physicist Feynman says to a physicist, the law of preservation means the existence of a number which is definable at a certain moment, and if we define it again after some time the number will not change—it will be of the same value, no matter what changes occurred in the meantime.

The threshold of the quick component was

diagnostic test that need be employed. Its results must always be supplemented by those obtained by chronaximetry; evaluation of the rheobase and electromyography.

Desynchronization of the response is often noted when the patient is examined more than 30 days after the onset of paralysis. The difference sometimes noted between the amplitude of the response and the extent of functional recovery is probably attributable to such desynchronization.

Like all previous authors in this field, we evaluate the amplitude of the potential only. Latency and the area of the wave are of scant diagnostic or prognostic interest.

We always soak our electrodes in saline, because the are flat and not of the suction-cup type.

When palsy is due to trauma, functional recovery may be noted a few days after the injury. This is the case when concussion is involved, since the phenomena to which it gives rise can be resolved in a few days if suitably treated, and does not represent an interruption of the nerve fibres. If the fracture site cannot be located radiographically, therefore, it is better to wait 15 days before embarking on surgery. This, in turn, will need to be undertaken after the 10th day when daily repetition of all the various electrodiagnostic tests has led to negative results for at least 4-5 days.

## THE R THRESHOLD OF THE QUICK COMPONENT

*A New Measure in Electronystagmography*

M. Pansini and I. F. Padovan

Zagreb, Yugoslavia

Vestibular imbalance affects the eyes through the fasciculus longitudinalis medialis and oculomotor nuclei. As regards vestibular excitation eyes could theoretically be turned around and around until balance is achieved. However, since physical and anatomical limitations do not allow such turning around, the eyes could be held in the utmost lateral position in the position of conjugated deviation. Nevertheless, the eyes are not held in this position of conjugated deviation since the compensatory central component brings the eyes back to the position of azimuth zero. Logically, this should depend on physical limitations and on the tension of the eye muscles which transmit information to the central structures via the proprioceptive afferent paths. The speed of the slow component and other elements also play a certain role here. It seems that we are faced with a great task of determining all these elements in order to define the point where the returning quick eye movement begins.

There are many elements which send commands to revert eyes. Sometimes the eye reverts after a high amplitude and sometimes after a low amplitude. It is not the same in all individuals (Collard & Eber, 1977). Many central mechanisms which are involved are not yet fully understood. The number of the unknown elements is too large to allow any possibility of their definition and measurement (Fig. 1).

However, the first step was carried out by determining the point where the slow component of nystagmus stops and the quick component begins as the point where the mechanism for reverting the eye starts to work. This point

could be called the threshold of the quick component. This means that the point is identified but not explained. Its identification makes it a possible subject for investigation.

*Identification and Measurement of R*

The assumption that this point can be defined only by knowing all the elements which determine it, is quite discouraging. It is possible, however, to make a comparison between the many elements of nystagmus which are measurable even though their mechanisms are still not well known. We may say that it is not absolutely necessary to know all the mechanisms in order to determine and measure this threshold. On the contrary, by making the threshold of the quick component measurable, it serves for purposes of investigation and learning about the elements which determine it.

The next step in our exploration was made when we assumed that if there is a certain threshold of sensitivity for the appearance of the quick component, then it must be constant in the examined individual, no matter whether nystagmus is weak or strong, whether it is at the beginning, in the middle or at the end of the provoked nystagmus. This is called the law of preservation. The Nobel Prize winner physicist Feynman says to a physicist, the law of preservation means the existence of a number which is definable at a certain moment and if we define it again after some time the number will not change—it will be of the same value, no matter what changes occurred in the meantime.

The threshold of the quick component was

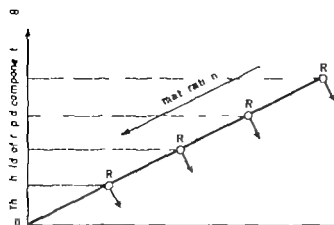


Fig 1

to be obtained as a numerical datum from already existing elements of nystagmus. In a nystagmic jerk we distinguish duration  $t$ , amplitude  $a$ , slow component  $f$  and quick component  $r$ . If as in geometry we mark sides with small letters and vertexes with capital ones, then the threshold of the quick component is the point  $R$  (Fig. 2).

After defining the threshold of the quick component and being convinced that  $R$  does not change in one measuring in a rather short time, it was possible to establish that  $R = s/f^2$  where  $s$  represents the speed of the slow component and  $f$  the frequency of nystagmus. In the actual nystagmogram it was shown that changes in frequency  $f$  and the speed of the slow component  $s$  do not cause significant changes in the threshold of the quick component  $R$ , which confirms the value of the above cited mathematical formula (Table I).

It was possible to confirm the value of the

Table I

| $f$ | $s$ | $R$ |
|-----|-----|-----|
| 3.1 | 25  | 2.6 |
| 1.7 | 6   | 2.1 |
| .2  | 11  | 2.3 |
| 1.6 | 4.5 | 1.7 |
| 1.3 | 3.5 | 2.1 |
| 0.9 |     | 2.5 |
| 1.2 | 2.8 | 1.9 |
| 3   | 19  | ~1  |
| 2.5 | 14  | 2.2 |
| 1   | 3   | 3   |
| 3.~ | 29  | ~8  |

formula on a model and also to show that the value for the threshold of the quick component has a span from zero to an indefinite number. It also shows a precision by which even small changes can be detected (Fig. 3).

Formula  $R = s/f^2$  worked well in practice although it was not clear what its basis was. It was hoped that mathematics, which is the foundation of all things and mechanisms, could sometimes solve such problems by itself. So it happened that by substituting  $a$  for  $s$  (from the old formula  $s = a \times f$ ) formula  $R = s/f^2$  transformed into  $R = a/f$  (Table II).

Now it was all very clear and simple. The threshold of the quick component will be greater if the amplitude is greater (it is directly proportional to the amplitude) and it will be smaller when the frequency is greater (it is conversely proportional to the frequency  $f$ ).  $R = a/f$ .

For practical purposes it is better to use formula  $R = s/f^2$  than the  $R = a/f$  and for easier calculation the following transformation can be used:  $R = (s/f) / f$ . The speed of the slow component is divided by the frequency and the result is again divided by the frequency which gives the value of the quick component.

In the 10 sec duration of nystagmus the number of nystagmic jerks is counted and divided by 10 which gives the value of frequency to one decimal place.

In the same section of the electronystagmogram we select three speeds of the slow component and calculate the average. The average

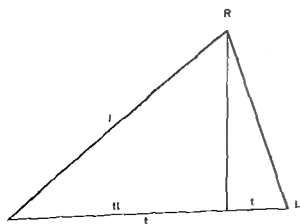


Fig 2

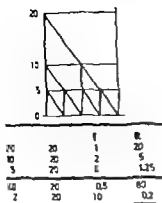


Fig. 3

of the slow component is then divided by the frequency and this result is again divided by the frequency according to the formula  $R = (s/f)/f$

#### Vestibular Maturity and R

We may ask if this datum has any meaning, considering the fact that it only measures the threshold of the quick component and does not determine the elements which caused it.

Table II

$$R = \frac{s}{f}$$

$$= \frac{s}{f}$$

$$\frac{1}{f}$$

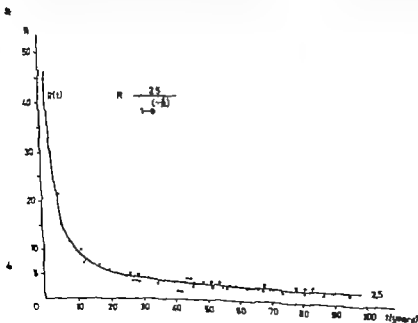
$$R = \frac{\frac{s}{f}}{\frac{1}{f}}$$

$$R = \frac{s}{f^2}$$

To defend the value of threshold of the quick component we list now some possibilities which are available for use

We took a large number of electronystagmograms of healthy individuals aged between 4 and 76 and measured R in each of the four caloric tests. Data were put on a graph where axis x carries the values for age and axis y the values of R (Fig. 4)

The line connecting the points which represent the average values shows a curve descending as age increases. This curve is regular in shape so we asked our electronics en-



gineer Mr Kozina to provide a formula for it which is as follows:

$$R = \frac{2.5}{1 - e^{(-\pi)}}$$

where

$R$  = threshold of the quick component

$e$  = natural logarithm

$t$  = age

This means that with increasing age  $R$  decreases and tends towards the value 2.5. When this formula was fed into the computer it produced values for age between 0 and 100 (Table III).

We obtained this curve and formula as well as the table on the basis of the specific number of examinations of healthy individuals which could differ in a new investigation. However we believe that mathematically the purest curve and formula would be the one which anticipates that  $R$  is infinite at the moment of conception (Tibbling 1969) while  $R$  is zero at the infinite age. Then we could say for the individuals with pathologically small  $R$  how many hundreds of years their results correspond to or we could calculate the  $R$  of Adam who lived 930 years or Methuselah the oldest man who lived 969 years.

When measuring  $R$  in certain individuals, we can say whether it corresponds his/her age taking of course into account certain physiological limits of  $\pm 20\%$ .

The threshold of the quick component certainly depends on myelination and generally on the maturity of the central nervous system, thus a bigger  $R$  in children suggests vestibular immaturity. We say vestibular immaturity because we think it refers primarily to the area of reticular formation and vestibular nuclei (Schmidt & Jeannerod 1970). It is also possible that there are cerebral influences from different regions, especially from the frontal and parietal areas, the cerebellum, limbic-limbic prefrontal and mesencephalic areas, as well as from the telencephalic fasciculus longus, the medulla and nuclei Caudal and Dorsal (see e.g. Raymond, 1971).

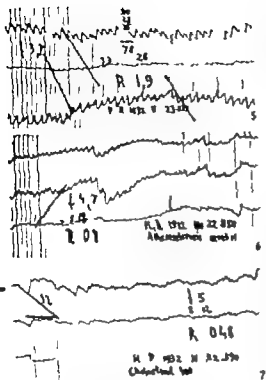
Table III

| $t$ (years) | $R$   | $t$ (years) | $R$  |
|-------------|-------|-------------|------|
| 0           |       |             |      |
| 1           | 88.76 | 51          | 3.26 |
| 2           | 45.01 | 52          | 3.13 |
| 3           | 30.43 | 53          | 3.20 |
| 4           | 23.15 | 54          | 3.18 |
| 5           | 18.78 | 55          | 3.16 |
| 6           | 15.87 | 56          | 3.13 |
| 7           | 13.79 | 57          | 3.11 |
| 8           | 12.44 | 58          | 3.09 |
| 9           | 11.03 | 59          | 3.07 |
| 10          | 10.06 | 60          | 3.05 |
| 11          | 9.27  | 61          | 3.03 |
| 12          | 8.61  | 62          | 3.01 |
| 13          | 8.06  | 63          | 3.00 |
| 14          | 7.57  | 64          | 2.98 |
| 15          | 7.17  | 65          | 2.96 |
| 16          | 6.81  | 66          | 2.95 |
| 17          | 6.50  | 67          | 2.93 |
| 18          | 6.22  | 68          | 2.92 |
| 19          | 5.97  | 69          | 2.90 |
| 20          | 5.74  | 70          | 2.89 |
| 21          | 5.54  | 71          | 2.88 |
| 22          | 5.36  | 72          | 2.87 |
| 23          | 5.19  | 73          | 2.85 |
| 24          | 5.04  | 74          | 2.84 |
| 25          | 4.90  | 75          | 2.83 |
| 26          | 4.77  | 76          | 2.82 |
| 27          | 4.65  | 77          | 2.81 |
| 28          | 4.54  | 78          | 2.80 |
| 29          | 4.44  | 79          | 2.79 |
| 30          | 4.34  | 80          | 2.78 |
| 31          | 4.25  | 81          | 2.77 |
| 32          | 4.17  | 82          | 2.76 |
| 33          | 4.10  | 83          | 2.75 |
| 34          | 4.02  | 84          | 2.74 |
| 35          | 3.95  | 85          | 2.73 |
| 36          | 3.89  | 86          | 2.72 |
| 37          | 3.83  | 87          | 2.71 |
| 38          | 3.77  | 88          | 2.70 |
| 39          | 3.71  | 89          | 2.69 |
| 40          | 3.65  | 90          | 2.68 |
| 41          | 3.60  | 91          | 2.67 |
| 42          | 3.55  | 92          | 2.66 |
| 43          | 3.50  | 93          | 2.65 |
| 44          | 3.45  | 94          | 2.64 |
| 45          | 3.40  | 95          | 2.63 |
| 46          | 3.35  | 96          | 2.62 |
| 47          | 3.30  | 97          | 2.61 |
| 48          | 3.25  | 98          | 2.60 |
| 49          | 3.20  | 99          | 2.59 |
| 50          | 3.15  | 100         | 2.58 |

### Clinical case

From a child with leukoencephalitis, we obtained a  $P$  which was larger than the one that normally corresponds to his age (13 yrs). As

Methodology which could evaluate neurological in-  
ter has not existed till now



Figs 5-7 5 A case of siderosis, with  $R=1.9$  6 Another case of central arteriosclerosis ( $R=0.8$ ) 7 A patient whose first measured  $R$  value was 0.5. It was subsequently found that his cholesterol value was 600

the illness progressed  $R$  increased to reach 64 in few months.

#### Angiopathies and R

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#### Possibilities open to investigation

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cation is only the beginning of the work which in our opinion promises significant diagnostic possibilities

As an example for investigation we would like to mention a formula for fixational suppression

$$FS = \frac{NF(\text{non-fixation}) - F(\text{fixation})}{NF(\text{non-fixation})}$$

where usually the speed of the slow component is worked with but it is also possible to use the threshold of the quick component  $R$ . We did it while examining a few healthy individuals and obtained the same values for the fixational suppression in  $s$  and  $R$

Fixational suppression expressed in  $s$  was 85.1 and in  $R$  it was 85.7. It is interesting because in some pathological cases regarding fixational suppression there was no connection between values of the speed of slow component and the threshold of the quick component. It means that it is possible to discover disturbances in some mechanisms of fixational suppression and surely there is more than one

It is easy to construct a formula for calculating  $R$  in each individual nystagmic jerk. We adjusted it to the speed of the ENG sheet of 15 mm/sec while for the speed of 10 mm/sec we should substitute the number 10 for 15

$$R = \frac{S}{\left(\frac{15}{N}\right)} \quad \text{or} \quad R = \frac{S}{\left(\frac{10}{N}\right)}$$

$N$  = length of the nystagmic jerk, in mm

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This is a new datum which can help in the exploration of nystagmic irregularities where the change in threshold of the quick component is mostly involved

Various influences and effects on the threshold of the quick component can be explored: effect of sedatives, influence of the various levels of intelligence and motorics, relationship with neurological diseases especially



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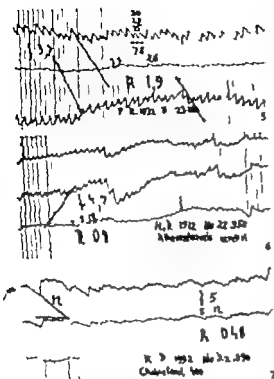
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Various influences and effects on the threshold of the quick component can be explored effect of sedatives influence of the various levels of intelligence and motoric relationship with neurological diseases especially

those causing demyelination and also the relationship between deafness and the rehabilitation of hearing

## CONCLUSION

Nystagmus is not a simple vestibulo-ocular reflex but one that passes through areas of the central nervous system which imprints its messages into provoked nystagmus. This is evident in an ENG recording. Reading those central messages and learning about this central semiology is the most significant area of electronystagmography today. We hope that  $R$ , the threshold of the quick component, will as a new measure also provide new possibilities for further explorations in this field.

## ACKNOWLEDGEMENT

My co-worker Professor M. Pansini began this investigation 4 years ago in our Department where our Laboratory for Vestibulology has gained extensive experience in electronystagmography. Our electronics engineer Mr. Kozma, assisted us greatly with our mathematical formulas and data processing. Our work is only preliminary but we can now confirm that these present results will not only be a subject for investigation but also be useful and applicable for routine clinical work.

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 I F Padovan M D  
 Vinogradskaja 29  
 Z greb  
 Yugo la ia

## DISCUSSION

Tonndorff to Padovan Nystagmus, as is generally known is a very noisy signal. Therefore a method of noise reduc-

tion is needed the simplest being averaging. Now with a time varying signal the time over which one averages—the time window—is important. It must not be too short but not too long either. What was your method of evaluation?

Jongkees to Padovan. What is the meaning of the term "threshold of the quick component" during a caloric test. It is certainly not so that the quick phase always reverts the eye to a mid-position. It can even take it further away from it.

Pfalz to Padovan. Your hypothesis is based on the results of repeated caloric tests. What parameter did you use to determine the threshold of a caloric response?

Manni to Padovan. I do not agree that the quick phase of eye nystagmus originates from conjunctiva receptors or from muscular proprioceptors. The source of the quick phase is located in the brain stem, mainly in the reticular formation. However the eye nystagmus may be influenced by trigeminal afferents.

### Padovan (Reply)

To Tonndorff. In 10 sec of the most regular nystagmus we determined the frequency, the three rates of slow component and then the average value of the slow component. No significant differences were observed in any of the 10 sec. The values of  $R$  were as a rule similar in four caloric tests. When we measured  $R$ , we measured it in all four or six caloric tests.

To Jongkees. We are interested in the phase the quick component begins. Whether the quick component of the eye approaches, reaches or passes the mid-position has an effect on the amplitude and a partial effect on the speed of the slow component which we observed in the recording. (However the correct speed of slow component is only when the eye reaches mid-position.)

All of these and other changes may have a significant effect on  $R$ , so that  $R$  is as unreliable as the amplitude, frequency and rate of slow component, which we likewise measure and consider important for the diagnosis.  $R$  is neither a more or less precise measurement than the others.

We call  $R$  the threshold of quick component, because a certain strength in the mechanism of the central component is necessary for the eye to return. This is not the same in each of the examinees. We call this reaction the threshold in the same way we do in the measurement of the stapedial reflex.

To Pfalz. In routine caloric tests we use the average value of the rate of the slow component obtained in 10 sec of the strongest response. We do not determine the threshold of the caloric response in the caloric test.

To Manni. We agree with the majority of authors that the quick phase originates in the reticular formation, that it is likewise influenced by trigeminal afferents and that many other influences certainly exist. However we do not consider these obstacles for determining and measuring  $R$  since we could reject all other measurements in electronystagmography for the same reasons. We have suggested  $R$  and its importance can be determined by all those who use it in their work.

## IMMUNODEFENCE OF THE INNER EAR?

*Lymphocyte-Macrophage Interaction in the Endolymphatic Sac*

Helge Rask Andersen and Jan Stahle

*From the Department of Otolaryngology, University Hospital, Uppsala*

**Abstract** Owing to their proximity to areas exposed to infection, the sensory organs of the inner ear are probably dependent on an efficient antimicrobial defence. The longitudinal flow of endolymph to the endolymphatic sac may be of major importance in this context. Substances entering the ear can be automatically carried to the distal part of the endolymphatic duct where lymphoid cells are present and endolymphatic phagocytosis occurs. In the latter distal part of the epithelium of the sac morphological signs marking the incoming substances are present. A vigorous interaction between lymphocytes and macrophages, similar to that observed in antigen-activated lymphoid tissue, may be seen. The sac is here surrounded by a rich network of lymphatic capillaries and blood vessels.

The endolymphatic duct and sac are believed to absorb endolymph. Guild stated in 1927 that endolymph flows from the cochlear duct to the endolymphatic sac where it is absorbed and mediated to the numerous perisaccular blood vessels.

Phagocytosis by both the epithelial cells and free cells of the endolymphatic sac was demonstrated experimentally by Andersen (1948), Engström & Hjort (1950), Arnvig (1951), Lundquist (1965) and Rudert (1967).

The purpose of a phagocytic cell system inside the endolymphatic space is not known. Does the sac receive from the labyrinth effete cells or metabolic waste products too large and toxic for disposal via the cochlear duct? Or do the freely floating cells cleanse the sac of proteins normally concentrated therein and transport them via the epithelium into the surrounding blood vessels, as proposed by Ishii et al. (1966).

Guild defined twenty-two species of cell element in the lumen of the endolymphatic

sac. These included lymphocytes whose presence prompted Linn & Silver's (1974) proposal that the endolymphatic sac acts as an immunodefensive organ for the inner ear. Since toxins or foreign substances could reach the labyrinth through the windows of the middle ear cavity it could be crucial to inner-ear homeostasis that these substances are removed and eliminated by phagocytic cells in the presence of immune cells.

In the present investigation lymphocytes and macrophages in the endolymphatic sac of guinea pigs were studied ultrastructurally. This was done in order to reveal a possible physical interaction which would indicate functional cooperation and could thus confirm the theory that the endolymphatic sac acts as an immunodefensive organ for the internal ear.

## MATERIAL AND METHODS

Fifty pigmented guinea pigs ranging in weight from 200 to 800 g were used in this investigation. The animals displayed a normal Preyer reflex. They were anaesthetized with sodium pentobarbital (Nembutal) and perfused with a solution of 2.5% (v/v) purified glutaraldehyde and 1% (v/v) formaldehyde in a solution of 0.1 M Sørensen's phosphate buffer, pH 7.2-7.4. After decapitation the middle and internal ears were examined macroscopically for signs of infection. The operculum covering the endolymphatic sac was eliminated with a fine



Fig 1 Light micrograph demonstrating the intra-osseous ES in the guinea pig. A few free luminal cells are seen in the darkly stained endolymph of the proximal sac where the endolymph appears vacuolated. The number of free luminal cells is increased towards the rugose portion of the

sac where a large number of cells approach the epithelium and the non-vascularized epithelial process (NVP). Inset: higher magnification of the NVP clearly reveals its unique structure as compared with the rest of the epithelium. Note its close association with the free luminal cells.

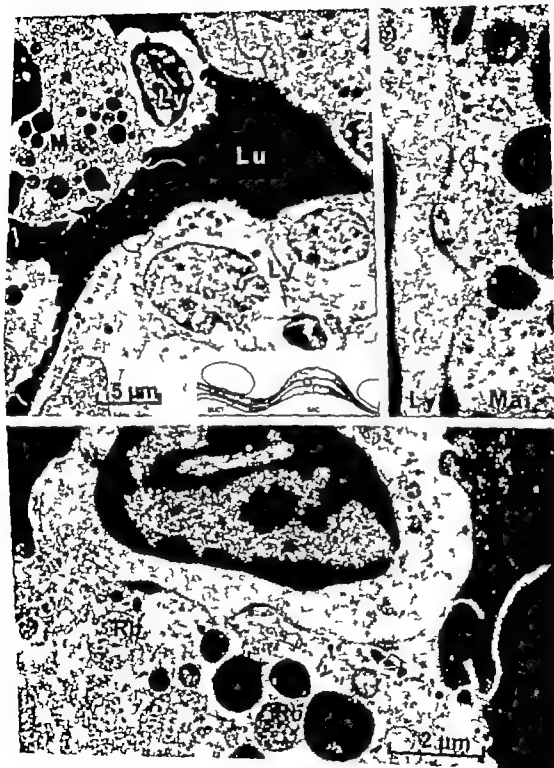
needle and the endolymphatic sac (ES; specimens displayed and dissected from the temporal bones). The specimens were immersed in the same fixative for 24 h post fixed in 1%  $\text{OsO}_4$ , dehydrated in alcohol and propylene oxide, embedded in Epon 812 and trimmed and sectioned on an LKB ultratome. Ten temporal bones were decalcified in 0.1 M Na EDTA and 2.5% purified glutaraldehyde in Na-cacodylate buffer for 14 days. The bones were serially sectioned both parallel and perpendicular to the long axis of the endolymphatic duct and sac. Semi-thin sections (1–2  $\mu\text{m}$ ) were stained with toluidine blue or *p*-phenylenediamine while ultrathin sections were contrast stained with uranyl acetate and lead citrate. The specimens were examined and photographed in a JEOL 100 B electron microscope.

## OBSERVATIONS

Earlier research on the vascular anatomy and supply of the endolymphatic duct and sac

(Rask Andersen 1979) revealed an extensive vascular network of arterioles, capillaries, venules and veins surrounding the endolymphatic sac while the endolymphatic duct was relatively poorly supplied with blood vessels. The endolymphatic duct is surrounded by a fairly dense network of lymph vessels which show a characteristic arrangement suggesting their participation in endolymph fluid transport (Rask Andersen & Bredberg 1979). These lymph capillaries lack a typical basal lamina and almost invariably display interendothelial gaps often facing the lumen of the endolymphatic duct. The lymph vessels surrounding

Fig 2 Top left: Two lymphoid cells in the intermediate portion of the ES. One lymphocyte (arrow) lies in the epithelium while another cell (Lv) is attached to the cell surface of a large intraluminal macrophage (Ma).  $\times 6300$ . Bottom: The lymphocyte adheres to the cell coat of the macrophage which forms cytoplasmic protrusions embracing the lymphocyte.  $\times 14500$ . The cytoplasm of the macrophage is filled with granular residual bodies (RB) and darkly stained organelles. Top right: Higher magnification showing the close contact between the lymphocyte and macrophage. Note areas of subplasmalemmal densities.  $\times 14000$ .



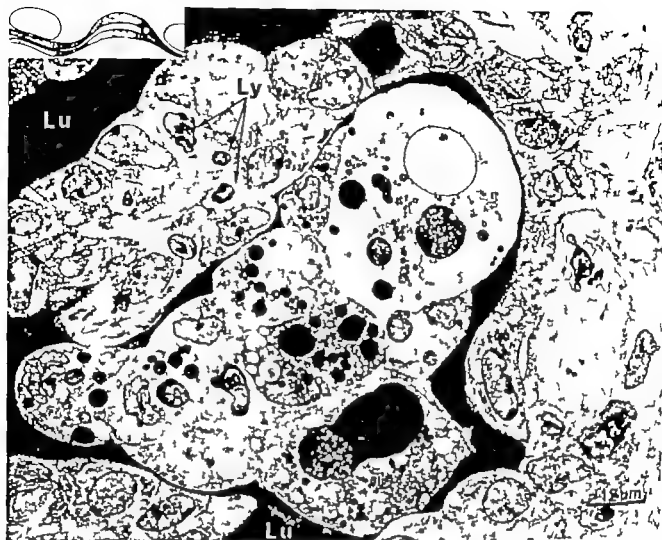


Fig 3 A cell cluster in the luminal space (Lu) approaches the villous epithelium of the rugose endolymphatic sac and is trapped in a deep crypt. The epithelium is infiltrated by

lymphoid cells (Ly). The cell cluster probably coalesces with the epithelium to form a non-vascularized epithelial process.  $\times 4900$

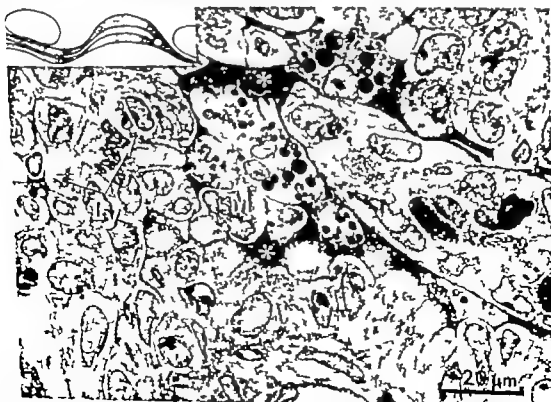
the endolymphatic sac are devoid of interendothelial gaps. Our studies suggest that the endolymphatic duct is concerned with fluid resorption while the sac is engaged in phagocytosis.

The proximal endolymphatic sac contains a viscous osmiophilic endolymph composed of clear vacuoles and particles. This precipitated endolymph is phagocytosed or engulfed by incoming macrophages or luminal histiocytes (LH) whose cytoplasm becomes filled with endolymph while the cells assume a signet ring appearance. Lymphocytes can be observed among these freely floating cells which later coalesce to form cell clusters. Many degenerate and appear to be transported distally in the endolymphatic sac (Fig. 1).

#### Lymphocyte-macrophage interaction

Macrophages and lymphocytes are intimately associated in the luminal space. The mac-

Fig 4 Electron micrographs of the rugose portion of the ES. (A) An epithelial cell process (NVP) project into the electron-dense endolymph which houses a few macrophages. The NVP is bordered by an irregular epithelium the cell cytoplasm of which often appears vacuolated. Among the epithelial cells are scattered polymorphonuclear leukocytes (PVN), a few monocytes (M) and numerous macrophages containing a number of granular residual bodies. The cells are closely apposed to each other. Adjacent columnar cells show fragmentation of the apical cytoplasm, and cytoplasmic blebs have discharged into the endolymph.  $\times 1500$ . (B) The dark endolymph contains huge binucleate amoeboid cells showing phagocytic properties. A lymphocyte (Ly) (left) migrates into a narrow epithelial crypt while another cell (right) lies close to the cell surface of a macrophage (Ma).  $\times 1100$ .





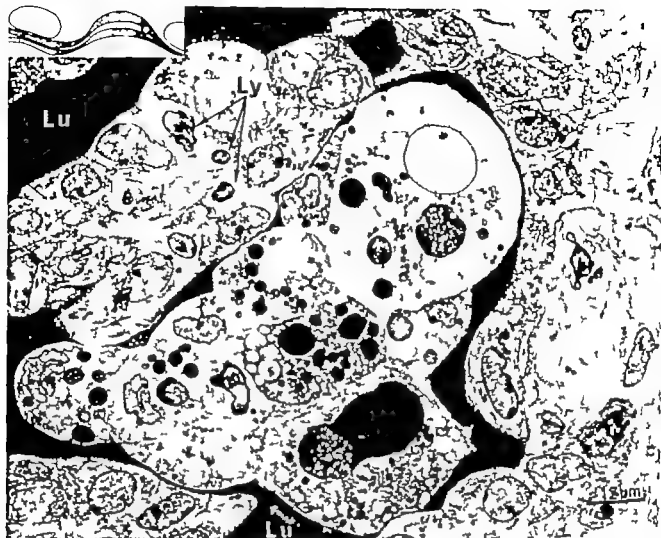


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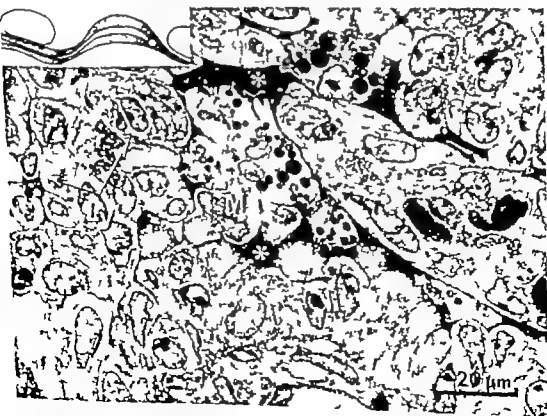
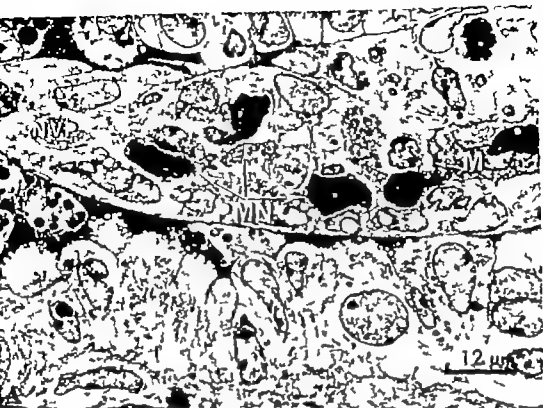
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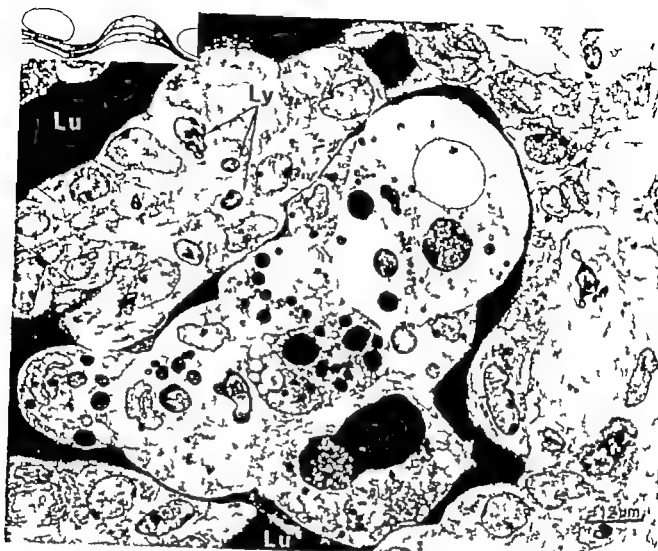


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Fig. 6. Lymphocytes (L) in the rugose epithelium of the ES. Top. The cells adhere to the epithelial cells (Ep) and display two nucleoli (N). Lr. Endolymphatic lumen. 2,400 (left),  $\times 1,750$  (right). Bottom. Pseudopodial

processes project into the cytoplasm of the epithelial cells. There are small areas of subplasmalemmal density  $\times 9,800$  (left),  $\times 5,400$  (right).

area of the intermediate portion of the sac were described with the help of light microscopy by Guild (1927) and ultrastructurally by Rask Andersen & Stahle (1979). They seem to be created when freely floating cell clusters fuse with the rugose epithelium (Fig. 3). They are rich in monocytes, macrophages and lymphocytes which probably originate from the perisaccular blood vessels. The epithelial crypts trap the cell cluster while the epithelium is invaded by mononuclear cells which later

are presumably involved in the degradation of the luminal cells (Fig. 4). Lymphocyte-macrophage interaction is a common finding in these NVP (Fig. 5). The macrophages contain secondary lysosomes filled with digested material. Their large nuclei display numerous prominent nucleoli. There are broad areas of close contact between the macrophages and lymphocytes. The lymphoid cells sometimes penetrate deep into the cytoplasm of the macrophages.

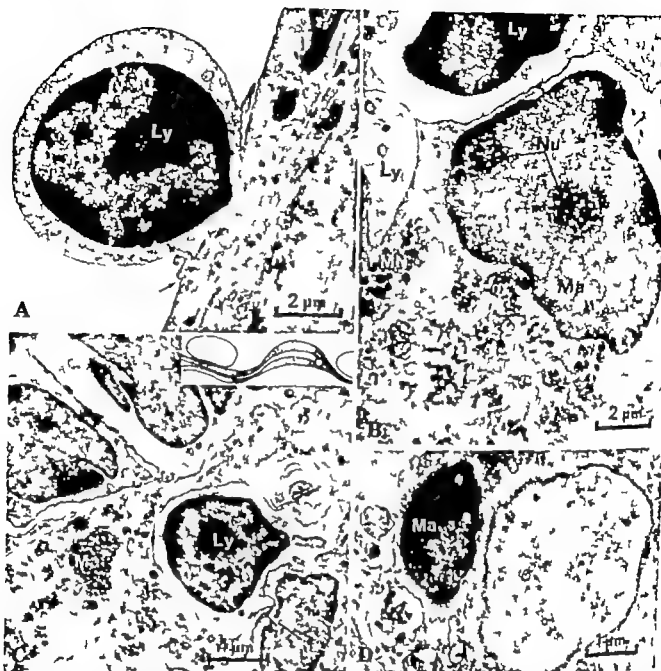


Fig. 5 (A) A lymphocyte (Ly) migrates through the thin endothelial cell of a post-capillary venule into the loose stroma around the rugose wall of the ES. Endothelial cell junction (arrow)  $\times 6600$  (B) Macrophage-lymphocyte association in a NVP of the ES. The macrophage contains numerous residual bodies. The large nucleus has two nucleoli (Nu). There is a broad area of close contact between the cells  $\times 9000$  (C) The lymphocyte has penetrated deep

into the cytoplasm of a macrophage (Ma) which is rich in intracytoplasmic residual bodies. A small uropod-like cytoplasmic protrusion of the lymphocyte is oriented peripherally  $\times 3100$  (D) One cell in a NVP has penetrated deep into the cytoplasm of a macrophage (Ma) and appears to be located intracellularly due to oblique sectioning. Rb=Residual body  $\times 11170$ .

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*Non vascularized epithelial processes (NVP)*  
NVP could be observed in all animals investigated. These epithelial processes in the distal

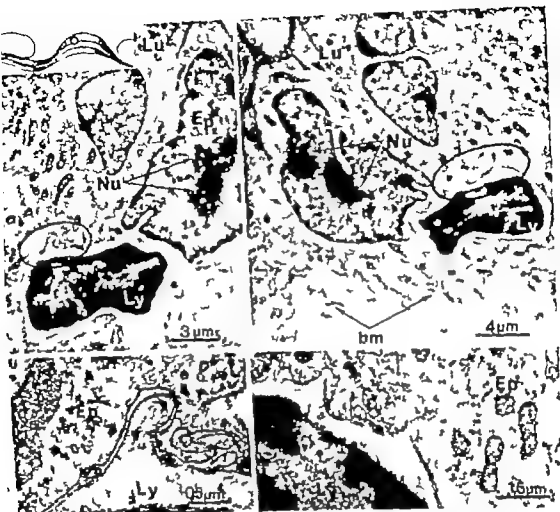


Fig. 6. Lymphocytes (Ly) in the rugose epithelium of the inner ear. Top: The cells adhere to the epithelial cells (Ep) and display two nucleoli (Nu). Lr: Endolymphatic space.  $\times 2400$  (left),  $\times 1750$  (right). Bottom: Pseudopodial

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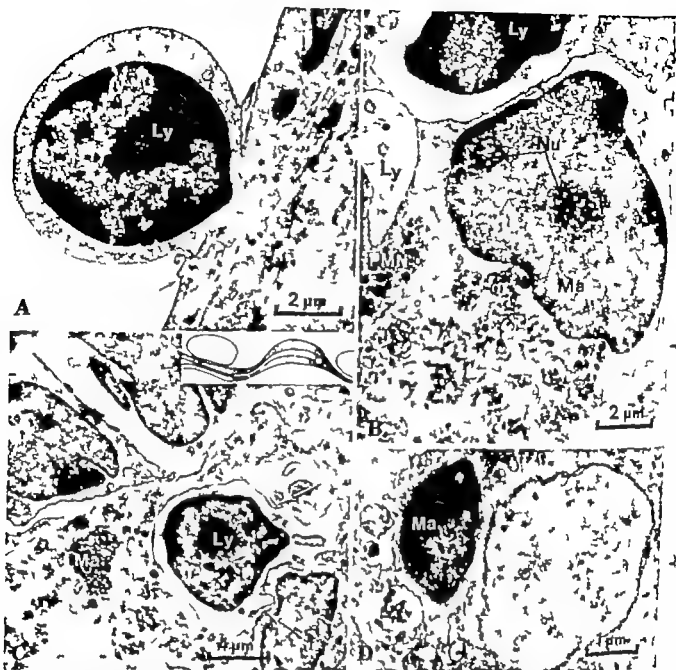


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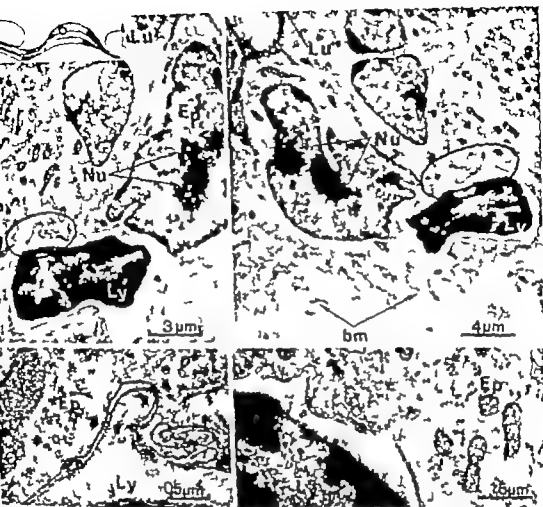


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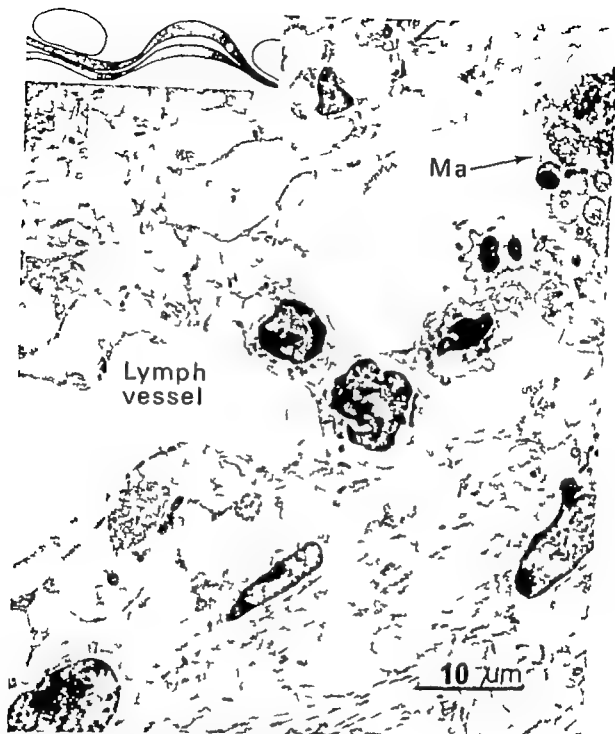


Fig 7 A lymph vessel situated between the intermediate and distal portions of the ES. It contains large lymphocytes and macrophages filled with condensed material

(Ma) The slender lymph vessel lacks typical basement membrane  $\times 2000$

### *Lymphocytes and sac epithelium*

Lymphocytes are found in the stroma surrounding the intra-osseous sac. Many take on a more primitive or blastoid appearance with a large translucent cytoplasm, their nuclei frequently containing a prominent nucleolus. Some cells appear to migrate into the

epithelium and assume a position close to the dark columnar epithelial cells (Fig 6). The nuclei of the dark cells adhering to lymphocytes display conspicuous nucleoli and in places the plasma membranes are thickened into what appear to be contact sites between the cells. No cytoplasmic bridges are found



Fig. 4. Plasma cells and lymphocytes in the distal region of the sac. This region of the perisaccular tissue is so rich in plasma cells in some animals that it may be called the 'plasma-cellular' region of the ES. *Top left*: Two mature plasma cells (PC) contain large amounts of rough endoplasmic reticulum and characteristic prominent perinuclear Golgi structures. Lymphoid cells appear as 'blasted' cells

and are scattered nearby. 1200. *Top right and bottom left*: The plasma cells (PC) have close relationship to the reticular connective tissue cells (R). Numerous coated pits and vesicles suggest macromolecular transport in the reticular cell cytoplasm. 5000. 9400. *Bottom right*: Plasma cells (PC) surround large lymphocytes. Prominent mitochondria and multivesicular body are seen. 7900.

#### Perisaccular tissue

The intra-osseous portion of the endolymphatic sac is surrounded by an extensive network of post-capillary venules and veins. It is not un-

usual to observe small lymphocytes migrating through the thin endothelial walls into the loose tissue (Fig. 5A). Mast cells are widely distributed, many of them with pseudopods

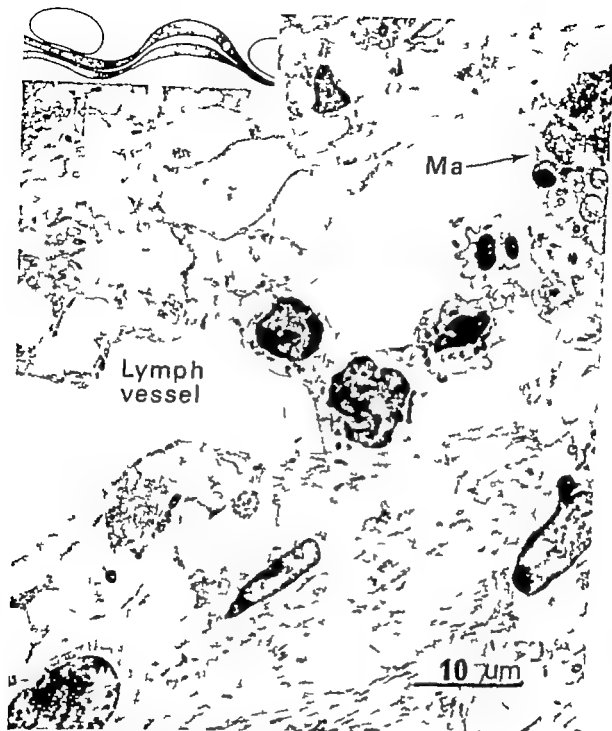


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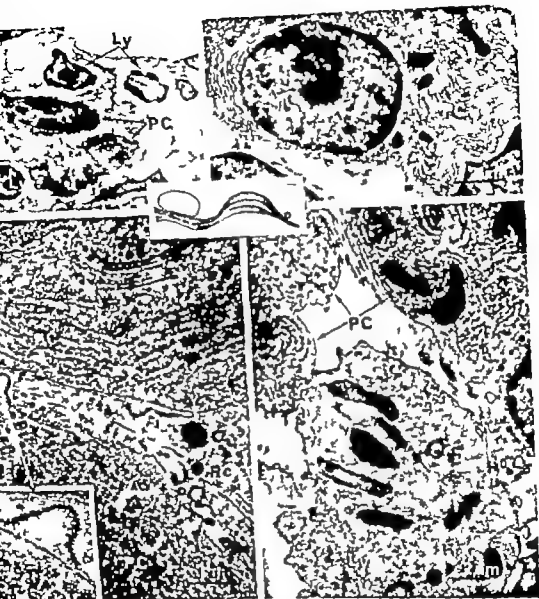


Fig. 8. Plasma cells and lymphocytes in the distal region of the sac. This region of the perisaccular tissue is so rich in plasma cells in some instances that it may be called the plasma-cellular region of the ES. *Top left*: Two mature plasma cells (PC) contain large amounts of rough endoplasmic reticulum and characteristic prominent perinuclear Golgi structures. Lymphoid cells appear as 'blastoid' cells

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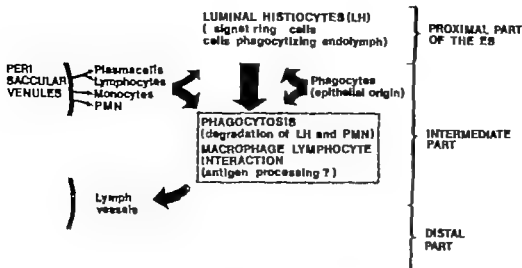


Fig 9 Hypothetical representation of the turnover of the free cells of the ES based on morphological observations in guinea pigs. Intraluminal cells start to appear in the distal endolymphatic duct and proximal sac. The cells ingest endolymph and assume a signet-ring appearance. More distally many cells form cell clusters and present a more degenerated appearance. Lymphocytes migrate through the thin walls of the perisaccular venules and appear around the luminal histiocytes (LH). Plasma cells, monocytes and polymorphonuclear leukocytes (PMN) are observed in the subepithelial connective tissue. Phago-

cytes originating from the intermediate sac epithelium are dispelled into the lumen to join the luminal histiocytes in the intermediate sac the epithelium is specialized into NVP where PMN and luminal histiocytes are being phagocytized. A close topographical relationship between macrophages and lymphocytes suggests the presence of an antigen-processing mechanism, while the region between the intermediate and distal sac incorporates a well-developed lymph capillary network, often containing both macrophages and lymphocytes, which probably indicate a continuous recirculation of lymphoid cells.

Dendritic melanocytes rich in spherical melanosomes and stellate reticular cells are scattered among reticular fibres, monocytes, polymorphonuclear leukocytes and plasma cells.

The endolymphatic sac is also surrounded by a prominent plexus of lymph vessels. These slender vessels lack a typical basement membrane and are often filled with lymphoid cells and macrophages containing condensed intracytoplasmic material (Fig 7). The number of lymph vessels increases towards the distal region of the sac where a large plexus is found. They are often of the same size as the neighbouring venules and veins. The tissue around the NVP displays a conspicuously large number of mature plasma cells (Fig 8). They contain masses of endoplasmic reticulum with prominent Golgi complexes. There are plasmoblasts and lymphoblastoid cells. Indeed by reason of the large amount of plasma cells the region near the external aperture of the vestibular aqueduct could be called the plasmacellular area of the ES.

## DISCUSSION

Macrophages are found in many organs of the mammalian body and many probably originate from the bone marrow through the migration of monocytes from the circulation into the tissue where they are transformed into macrophages and even epithelioid cells and giant cells. Nowadays considerable attention is focused on the macrophages during the immune responses. Macrophages are able to trap and process antigen and appear to play an essential part in the presentation of antigen to immunospecific lymphocytes before the antigen response can become manifest (Cline & Swett 1968; Hersh & Harris 1968). Such functional co-operation may include intimate contact between the participating cells. Morphological studies have demonstrated physical interaction between lymphoid cells in various lymphatic tissues (Sharp & Burwell 1960) and direct cytoplasmic bridges between lymphocytes and macrophages have been observed after challenge with antigen (Schoenberg et al 1964). In the ES in guinea pig macrophage

lymphocyte associations are observed in both the luminal space and the NVP of the intermediate portion of the endolymphatic sac epithelium. Such associations are apparently not merely a rare coincidence of migrating lymphoid cells but represent actual physical interaction due to intercellular proximity. Presence of nucleoli and differentiation of the plasma membranes showing unspecific contact sites. Thus may represent functional co-operation between the cells. It is not known whether the cells are involved in any specific antigen-processing mechanism or immune response and this question needs to be investigated further. The close relationship between lymphoid cells and dark epithelial cells is interesting. According to Lundquist (1965) the dark cells of the intermediate sac are potential phagocytes which can probably be dispelled into the lumen to become freely floating cells. It is tempting to speculate that lymphocytes may interact also with epithelial cells before they differentiate into phagocytic cells.

An intricate complex interaction between immune epithelial and phagocytic cells may in future be proved to promote phagocytosis in the ES.

On the basis of our morphological studies we devised a hypothetical model of the turnover of some of the free cells of the endolymphatic sac (Fig. 9). We postulate in principle two different kinds of phagocytic cell systems in the sac. One is composed of free cells (LH) appearing in the proximal sac which often assume a signet-ring appearance. The cells ingest large quantities of viscous endolymph and seem to be endolymph drinkers. The cells move distally to form cell clusters. After they have been trapped by the villous epithelium of the intermediate sac these aggregations of cells encounter a second system of free cells derived from the blood stream. This consists of lymphocytes, monocytes and polymorphonuclear cells (PMN). The luminal histiocytes and PMN cells are degraded in the NVP in which are scattered monocytes and lymphoid cells. Free cells may also emanate from the epithelium of the intermediate sac. It is suggested

that processing of antigen with the activation of lymphocytes occurs in the sac lumen and in the NVP. These cells together with macrophages containing concentrated digested material recirculate through the extensive network of lymph vessels surrounding this area of the ES. Some lymphocytes undergo differentiation into plasmoblasts and plasma cells.

## ZUSAMMENFASSUNG

Die Sinnesorgane des Innenohrs mit ihrer Nähe zu infektiösen ausgedehnten Bereichen sind wahrscheinlich abhängig von einer wirksamen offenen antimikrobiellen Verteilung. In diesem Zusammenhang kann der sogenannte longitudinale Strom der Endolymph in dem Saccus endolymphaticus eine große Rolle spielen. Eindringende Stoffe können automatisch abtransportiert werden. Im distalen endolymphatischen Gang treten lymphoide Zellen und dunkle Phagocyten der Endolympe auf. Im intermediären Abschnitt des Saccul epithel treten morphologische Anzeichen einer Kodifizierung der einfließenden antigenen Substanzen zutage. Hier findet auch eine lebhafteste Aktivität der Lymphocyten und Makrophagen miteinander statt, wie man sie auch in antigenaktivierten lymphoiden Geweben sehen kann. Hier befindet sich auch ein dichtes Netzwerk von Lymphkapillären und Blutgefäßen.

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## DISCUSSION

*Albeger to Stahle* Firstly I would like to congratulate you on the excellent morphological study and the interesting results. In this connection I want only to stress two points

1. We have also found the same cell clusters in the human nasal and paranasal sinus mucosa, consisting of lymphocytes and macrophages. But in contrast to your findings we could not detect any specialization of the cell membrane at the contact zones or even fusion of the cytoplasm at the sites of contact.

2. The second point concerns the nature of these cell clusters. There is no doubt that such a cluster formation is common during the immunization process, thus favoring intercellular cooperation which may be necessary in some kinds of immune reactions. In subsequent experiments we found such cluster formations also in some other conditions without any immunization processes, for example in tissue cultures. Therefore we believe that the cluster formation may also be an unspecific and non-immunological phenomenon of immune-competent cells.

*Thalmann to Stahle* I would like to ask you about your estimate of bulk flow of endolymph towards the endolymphatic duct.

*Harrison to Stahle* Has your material come from fresh human post-mortem specimens or the experimental animal? On how many observations is your experience based?

*Wersäll to Stahle* The quality of the morphological study was very high. Have you analysed the quantity of active mononuclear cells by means of immunofluorescence in various parts of the labyrinth? Is there an even distribution or are they concentrated around the sac?

*Sarfen Jr to Stahle* It was interesting to see the accumulation of intraluminal cells. Do you know what happens with these cells?

*Jakobi to Stahle* Following your results—is it too easy to protect the inner ear by antibiotics in sterile operations, as in cases of otosclerosis?

*Rask-Andersen and Stahl (Reply)*

*Rask-Andersen to Albeger:* In our study we could not perceive direct cytoplasmic bridges between lymphocytes and macrophages such as were earlier demonstrated in various lymphatic tissues by Schoenberg et al. This may be because the lymphoid cells in the sac are few in relation to lymphoid tissue and these direct intercellular communications are of short duration. We do not know whether the physical interactions between cells in the endolymphatic sac reflect specific immune reactions in progress in the sac but this matter should be investigated further.

*Rask Andersen to Thalmann* No volumetric measurements have been performed but morphology may indicate a considerable resorption in the duct.

*Rask-Andersen to Harrison* We have examined almost 100 guinea pigs. All the animals displayed non-asymmetrized epithelial processes but there were considerable variations in the number of freely floating cells in the sac.

*Rask Andersen to Wersäll* We have performed immunofluorescence in order to identify the lymphocytes in the perimacular tissue. We found labelled cells in this tissue but also in the epithelium in the luminal space and especially in the NVP which suggests that a substantial number of the lymphocytes in the sac may belong to the B-lymphocyte population.

*Stahle to Portmann* A deficient pressure regulation capacity of the duct and sac can be due to changes in the epithelium as well as in the vascular transport mechanism. Our finding of a rich network of lymph vessels surrounding the distal part of the endolymphatic duct may indicate that water reabsorption takes place mainly in this region. This specific function may be disturbed by periductal fibrosis or formation of new bone resulting in obstruction of the endolymphatic duct.

*Rask Andersen to Sarfen Jr* The freely floating cells are degraded and phagocytosed by macrophages entering from the perimacular blood vessels as monocytes. The cells concentrate in their cytoplasm indigestible cellular material which is most obviously seen when they recirculate through the lymph vessels.

*Stahle & J. Loh* Our results indicate a built-in immune-defence organ in the endolymphatic compartment. This might have shielded us from having labyrinthitis in childhood. As far as otosclerosis surgery is concerned I never use prophylactic antibiotic treatment. Our recent findings can hardly be used as an argument when discussing the value of prophylactic antibiotic treatment.

## OBLITERATION OF THE DUCTUS REUNIENS

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and Diane D. Jones

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**Abstract.** The ductus reuniens was successfully obliterated in 51 guinea pig ears. Histopathological study showed that majority of these specimens demonstrated cochlear hydrops, saccular collapse and normal utricle. These results support the theory of longitudinal flow of endolymph from the cochlea toward the endolymphatic sac via the ductus reuniens and sacculus. A major source of endolymph in the sacculus appears to be the scala media. In another set of 11 animals in which the ductus reuniens was first obstructed and two months later the endolymphatic duct was blocked endolymphatic hydrops as shown in the cochlear saccules, and utricle of all but one. The evidence suggests that cochlear hydrops was caused by obliteration of the ductus reuniens, and the saccular and utricular hydrops occurred subsequently as the result of blockage of the endolymphatic duct. Remnants of otolithic membranes which were attached to the distended saccular wall indicate that the membrane which had collapsed onto the staculus after obliteration of the ductus reuniens is capable of subsequent distension. This experiment supports the concept of endolymph flow from the utricle and canals toward the endolymphatic sac. A blocked ductus reuniens might also explain the pathophysiological basis for the auditory form of Meniere's disease.

There are two principal theories concerning endolymph secretion and absorption in the membranous labyrinth. In the longitudinal flow theory endolymph of the cochlea is produced in the scala media and passes through the ductus reuniens, saccule and endolymphatic duct to reach the endolymphatic sac where absorption takes place (Guild 1927, Lundquist 1965). Guild (1927) postulated that in the vestibular system there is a flow of endolymph from the utricle and ampullae through the utriculo-endolymphatic valve toward the endolymphatic sac. The radial flow theory contends that endolymph is both produced and absorbed locally within the scala media (Naftalin & Harrison 1958, Lawrence & Mc

Cabe 1959, Lawrence et al. 1961, Lawrence 1966) as well as in the utricle (Dohman 1964, 1965). The radial flow theory appeared to gain support from reports by Lindsay (1947) and Suh & Cody (1977) that no change in membrane position occurred after obliteration of the endolymphatic sac in the monkey and chinchilla respectively. On the other hand the occurrence of consistent endolymphatic hydrops in other species of mammals after obliteration of the endolymphatic duct or sac (Kimura & Schuknecht 1965, Kimura 1967, 1976, Schuknecht et al. 1968, Beal 1968 and others) supports the longitudinal flow theory.

Since the results of these experiments are clearly in conflict, an experiment was designed to further test the validity of the radial or longitudinal flow theory in the guinea pig which is ideally suited for this type of experiment. The experiment consisted of morphological studies on two groups of guinea pigs: one with obstruction of the ductus reuniens alone and the other with obstruction of the ductus reuniens followed later by obstruction of the endolymphatic duct. The findings might also have relevance concerning individual dependence of the auditory and vestibular labyrinths on the absorptive function of the endolymphatic sac. Furthermore, the experiment might shed light on the pathophysiology of atypical forms of Meniere's disease. It is known that endolymphatic hydrops is the principal pathological feature of this disease and that most affected patients will exhibit both auditory and vestibular symptoms. Atypical cases do occur, however, where the symptoms and functional test



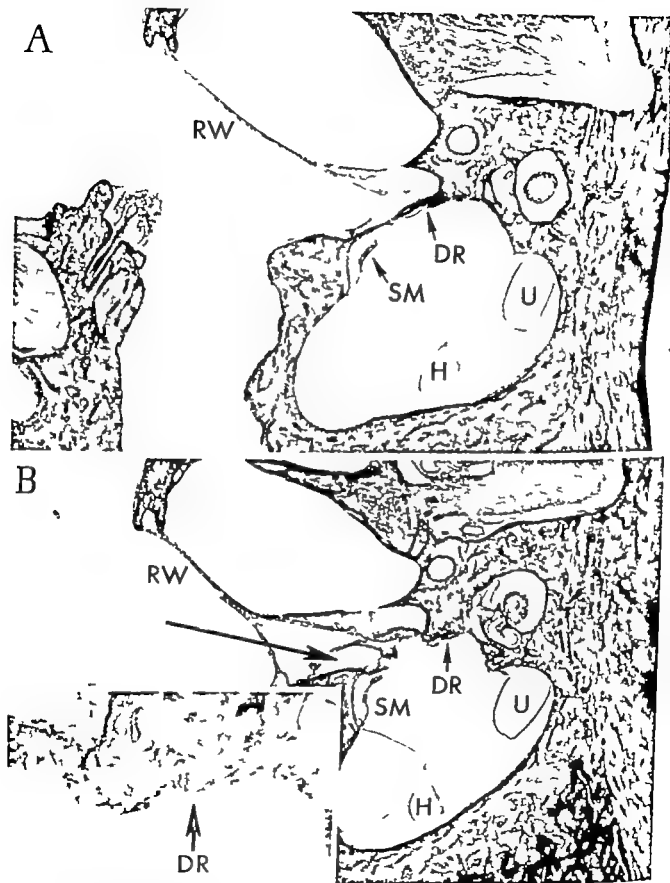


Fig. 1 (A) The ductus reuniens (DR) is shown at the entrance of the scala media (SM) in a normal specimen. The ductus reuniens was followed to the saccule in serial sections. (B) The ductus reuniens was obliterated through

the direction of the curved needle inserted to cut the ductus reuniens. The inset shows a higher magnification of the obliterated ductus reuniens (DR). The same specimen is shown in Figs. 2 and 3. U, Utricle; H, horizontal

Table 1

|  | Round<br>window<br>approach<br>(35) | Ves-<br>tibule<br>approach<br>(17) | Total<br>(52) |
|--|-------------------------------------|------------------------------------|---------------|
| Cochlear hydrops<br>Saccular collapse<br>Utricule normal     | 3                                   | 1                                  | 4<br>(67%)    |
| Cochlear hydrops<br>Saccule normal<br>Utricule normal        | 6                                   |                                    | 6<br>(115%)   |
| Cochlear hydrops<br>Saccular collapse<br>Utricular hydrops   |                                     | 2                                  |               |
| Cochlear hydrops<br>Saccular collapse<br>Utricular collapse  | 1                                   | 1                                  |               |
| Cochlea normal<br>Saccular collapse<br>Utricula normal       |                                     |                                    | 1             |
| Cochlear collapse<br>Saccule normal<br>Utricule normal       | 1                                   |                                    | 1             |
| Cochlear collapse<br>Saccular collapse<br>Utricular collapse | 2                                   |                                    | 2             |

deficits are either auditory or vestibular but not both.

The experiment was based on the assumption that the reparative response to surgical interruption of the ductus reuniens would cause obstruction of its lumen. Previous experiments have shown the propensity of the labyrinth to heal surgically induced fistulae (Kimura & Schuknecht, 1973; Kimura et al. 1977). If a longitudinal flow does occur then obstructing the ductus reuniens should cause expansion of the scala media by accumulation of endolymph. The saccule, on the other hand, should collapse or remain normal. If the endolymphatic duct were subsequently blocked the utricle, ampullae and possibly the saccule would also become hydropic. If there were only a radial flow no change would be expected in the endolymphatic spaces. In addition to the two sets of experiments appropriate control procedures were also performed. The studies are concerned principally

with the position of the membranous endolymphatic walls.

## MATERIALS AND METHODS

A total of 126 guinea pig ears were used for the entire experiment. This large number was required since the surgical approach to the ductus reuniens was blind and surgical complications were involved. In 88 ears only the ductus reuniens was obliterated by two different approaches: round window (63) and vestibule (25). In 11 ears the endolymphatic duct was blocked two months after obliteration of the ductus reuniens by the round window approach. In the remaining 26 ears three different control experiments were performed. In many guinea pigs both ears were operated in order to conserve time, space and effort.

The operative approach to the ductus reuniens was through the external auditory canal. Under aseptic conditions the tympanic membrane was perforated and the cochlear duct was visualized through the round window membrane. At the area close to the end of the cochlear duct a fine curved needle was inserted through the round window and basilar membranes and cuts were made by a scraping motion toward the osseous spiral lamina where the ductus reuniens enters into the cochlear duct. In 13 ears a stainless steel pin (465 µm) was pushed into the same area. The perforated round window membrane was covered with a small piece of gelfoam.

In order to test the validity of the results obtained from the round window approach another surgical approach to the ductus reuniens was made. The bony wall posterior to the round window was drilled open and the ductus reuniens was reached through the cistern of the vestibule by a long curved needle. Cuts were made at the predetermined area, at about the mid-length of the ductus reuniens. The surgical procedure for obliteration of the endolymphatic duct and sac was described earlier (Kimura & Schuknecht, 1965).

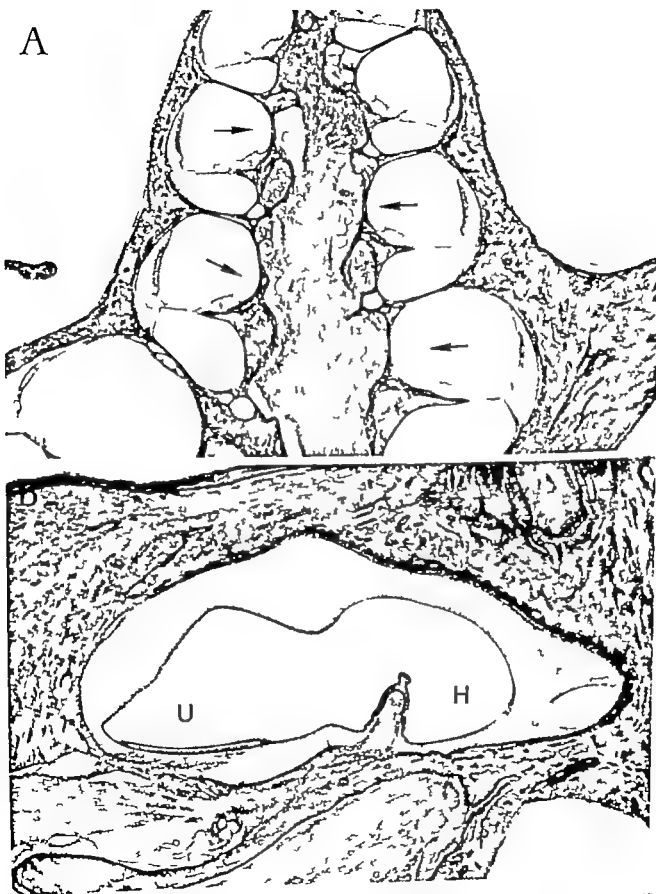


Fig 2 Both photographs are of the same specimen as shown in Fig 1B (A) The cochlea shows endolymphatic hydrops (arrows) resulting from obliteration of the ductus reuniens. (B) The utricle (U) and horizontal ampulla (H) remain normal

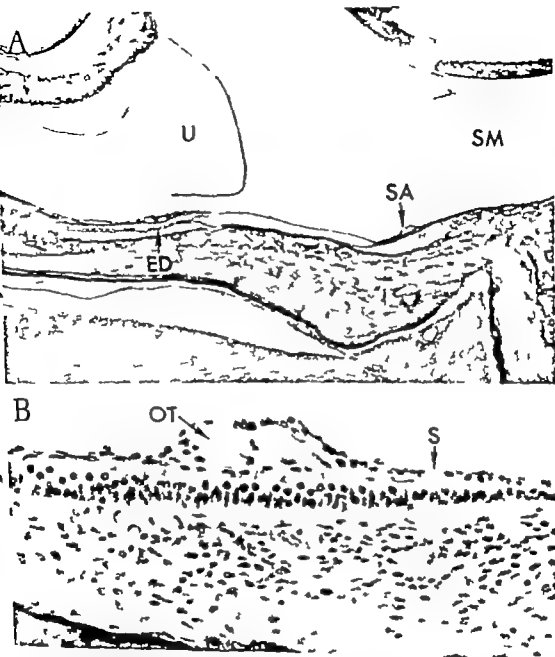


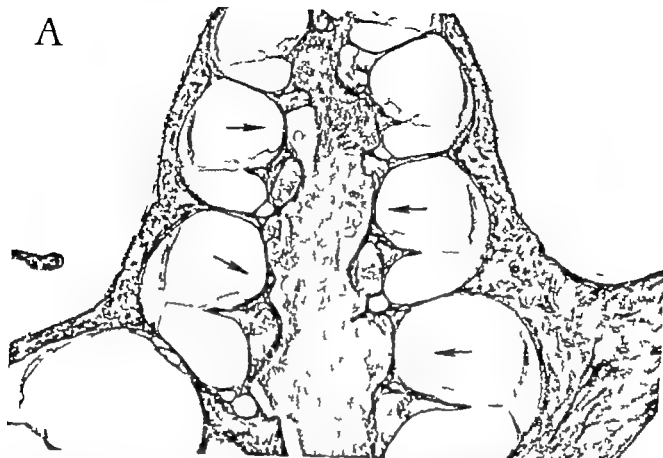
Fig. 3. The same specimen as shown in Figs. 1B and 2A. (A) The saccular membrane is collapsed after ductus reuniens obliteration. The degenerated saccular membrane (SM) is shown at the right. The endolymphatic duct (ED) is

normal and patent. SA: Saccule-Utricle. (B) A higher magnification of the saccus sacculi in Fig. 3A showing the degenerated otolithic membrane (OT). The mass is surrounded by the saccular membrane (S).

As controls of the experiment, the round windows were opened in six ears, the cochlear ducts were perforated through both Reissner's and basilar membranes in 13 ears and sta-

pedes were mobilized or subluxated in seven ears. The saccular perforations performed in nine ears of an earlier experiment (Kimura et al. 1977) served as another experimental con-

A



B

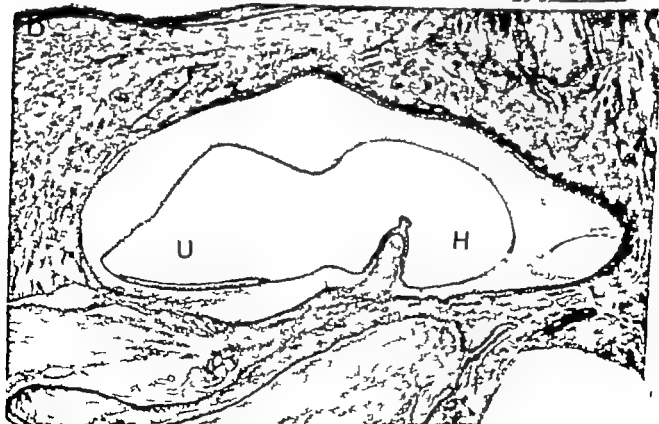
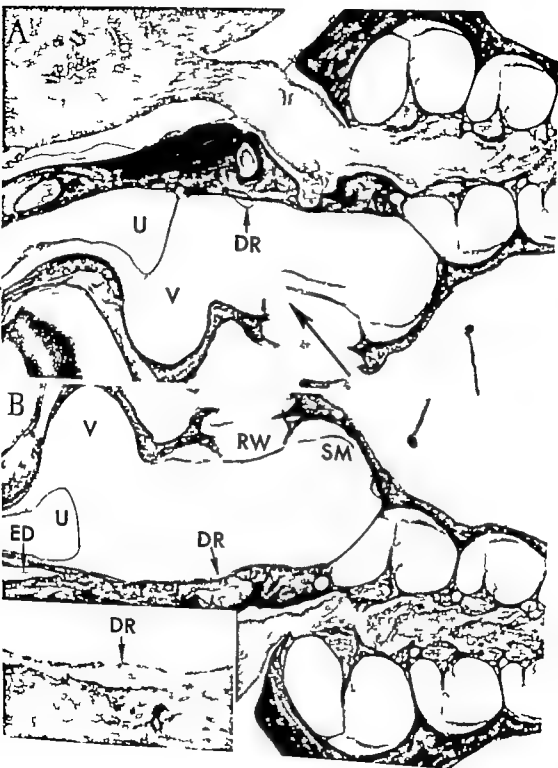


Fig 2 Both photographs are of the same specimen as shown in Fig. 1B (A) The cochlea shows endolymphatic hydrops (arrows) resulting from obliteration of the ductus reuniens. (B) The utricle (U) and horizontal ampolla (H) remain normal



|                            | Hydrops   | Collapse | Normal   |
|----------------------------|-----------|----------|----------|
| Round Window approach (35) |           |          |          |
| Cochlea                    | 30 (86%)  | 3        | 2        |
| Sacculle                   | 0         | 28 (80%) | 7        |
| Utricule                   | 0         | 7        | 78 (80%) |
| Vestibule approach (17)    |           |          |          |
| Cochlea                    | 17 (100%) | 0        | 0        |
| Sacculle                   | 0         | 15 (88%) | 2        |
| Utricule                   |           | 1        | 14 (82%) |
| Total (52)                 |           |          |          |
| Cochlea                    | 47 (90%)  | 3        |          |
| Sacculle                   | 0         | 43 (83%) | 9        |
| Utricule                   | 2         | 8        | 42 (81%) |

trol In addition 80 other normal or unoperated ears were studied as controls

The survival times of almost all the experimental animals in the present study were five months In animals in which two operations were performed the survival time was five months after ductus reuniens obliteration and three months after obliteration of the endolymphatic duct All animals were systemically perfused with Heidenhain-Susa fixative and the ears were processed by the routine celloidin technique for serial sectioning at 20  $\mu$  thickness Every tenth section was stained and examined In many specimens all serial sections were examined in the area where the ductus reuniens had been surgically obliterated

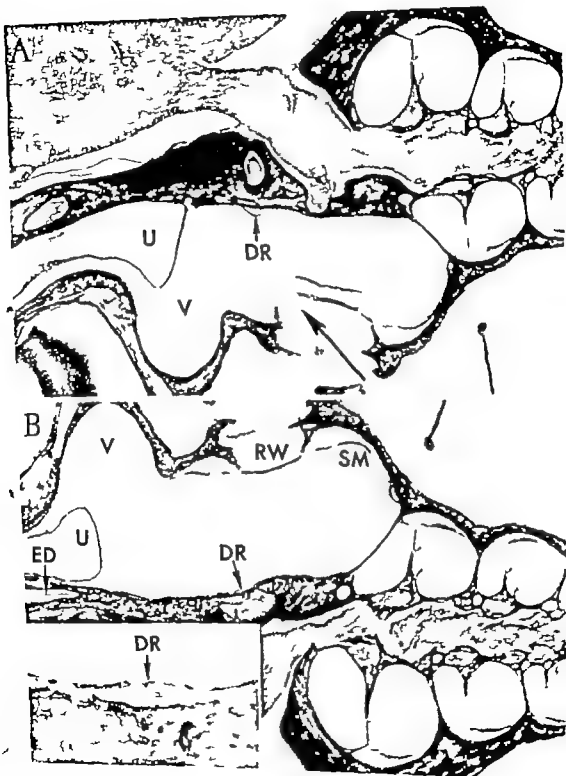
The histological appearance of the ductus reuniens was evaluated at the initial segment where trauma was introduced the area adjacent to the trauma, the mid point of the duct and the area close to the sacculle The ductus reuniens was judged as being completely absent (or atrophied) collapsed or normal The evaluation was made without prior knowledge of the pathological alterations in other parts of the membranous labyrinth In another separate evaluation the positions of the membranous walls of the cochlear and vestibular labyrinths were recorded without prior knowledge of the condition of the ductus reuniens These two sets of data were put together for

## FINDINGS

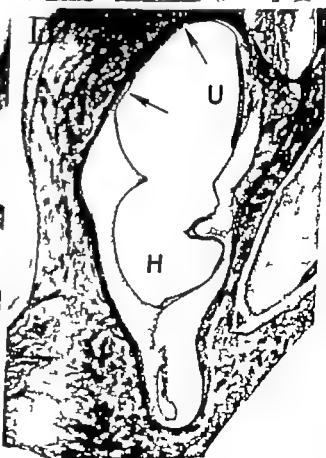
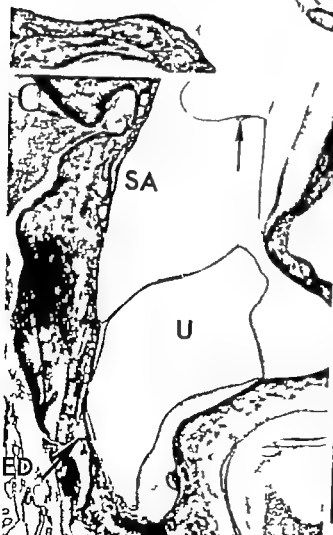
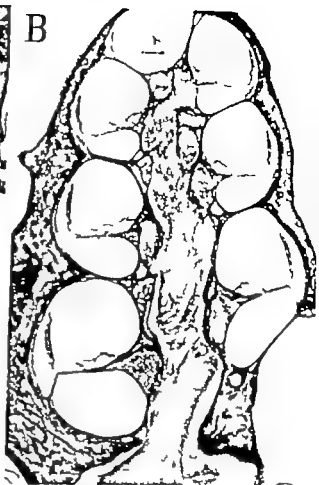
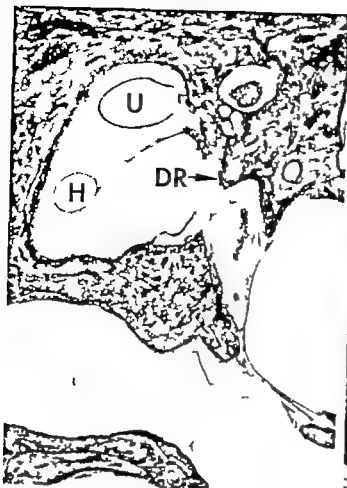
Although 89 ears were operated to interrupt the ductus reuniens histological studies revealed successful obliteration in only 52 (Fig 1) The remainder showed no obliteration partial damage or reconnection of the membranous duct or they demonstrated otitis media and/or inflammatory reaction concomitant with obliteration of the ductus reuniens When the positions of the membranous walls of the 52 inner ears were studied several different combinations of features became apparent (Table I) The most frequent combination was cochlear hydrops + saccular collapse and normal utricle which occurred in 35 out of 52 ears (67%) (Figs 2 and 3) The second most frequent combination was cochlear hydrops with normal sacculle and normal utricle (8 of 52 15%) When the positions of the membranous walls in each structure were compared the cochlea showed the highest incidence of endolymphatic hydrops (90%) the sacculle showed the highest incidence of collapse (83%) and the utricle was most frequently normal (81%) (Table II)

The approach to the ductus reuniens via the vestibule was more successful than via the round window Insertion of a pin into the cochlear duct near the ductus reuniens through the round window did not improve the rate of successful obliteration about 50% of 13 ears Although the approach through the

Fig 4 (A) The ductus reuniens (DR) is shown at a location close to the sacculle The lumen is open in spite of the fact that the scala media was perforated through the round window membrane (arrow) as a control Note the normal position of Reissner's membrane U Utricule V vestibule (B) The ductus reuniens (DR) was obliterated by the approach from the vestibule The operative site is not shown A higher magnification of the obliterated ductus reuniens (DR) is shown in the inset Note the cochlear hydrops ED Endolymphatic duct U utricle V vestibule RW round window SM scala media.







vestibule was a more certain method for obliterating the ductus reunientes (Fig. 4) the macula sacculi was often traumatized. Opening the bony capsule produced inflammatory reactions of fibrosis and ossification at the operative site and adjacent perilymphatic space. In some specimens the lumen of the endolymphatic duct became very narrow or closed or the utriculo-endolymphatic valve appeared to be closed, both of which occurred in association with endolymphatic hydrops of the utricle.

Histopathological alterations in the cochlea resulting from the round window approach were atrophy of sensory cells, spiral ganglia and striae vasculares in the basal and apical turns. The connective cells of the limbus often degenerated in corresponding areas. The sensory cells of the sacculi often became atrophic, particularly in the areas where the saccular membrane had collapsed on top of the degenerated otolithic membrane. It was common to see small remnants of the otolithic membrane encapsulated by cells which were similar to the cells of the saccular membrane (Fig. 3B). The utricles were normal. The ampullae were normal except for a few atrophic posterior cristae, possibly caused by trauma associated with inadvertent surgical perforation of the adjacent utricular wall.

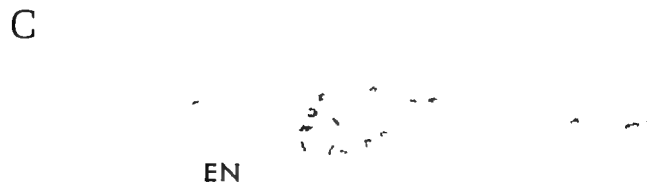
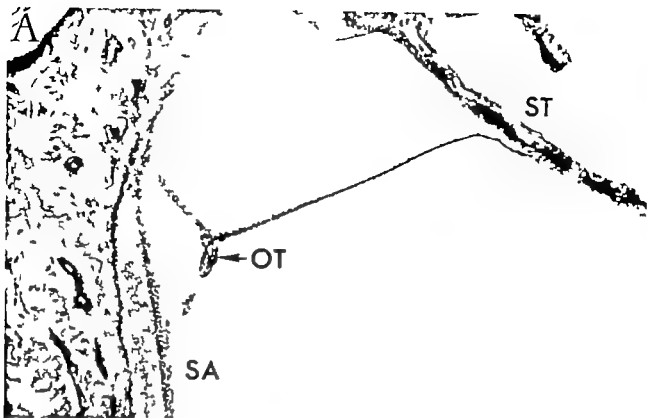
Histopathological changes observed in specimens with the vestibular approach were similar to those of the round window approach, although more severe sensory cell changes were seen at the extreme basal end of the cochlea where drilling occurred or where the spiral ligament was sometimes

traumatized. The saccules showed degenerated neuroepithelium often as the result of direct trauma. The utricles and most of the ampullae remained normal. There were atrophic changes in some of the ampullae of the posterior semicircular canals.

In almost all 80 normal control ears the ductus reunientes remained open. They were collapsed in a few specimens but were not obliterated or atrophic as observed in experimental ears. The positions of the membranous walls of the cochleae, saccules and utricles remained normal in a majority of the specimens. A slight distension of Reissner's membranes in the round window areas or a slight collapse of the utricles was sometimes noted. One specimen showed a diffuse collapse of the cochlea, saccule and utricle.

In the 13 control ears in which the round window membranes and cochlear ducts were pierced by a needle the ductus reunientes remained open in 12, all of which showed normal position of Reissner's membranes. One specimen showed an obliterated ductus reunientes due to accidental trauma which was associated with a slight distension of Reissner's membrane. Most of the saccules and utricles remained normal though a few showed slight collapse. In 11 experimental ears in which obliteration of the ductus reunientes failed the positions of the membranous walls of the cochleae, saccules and utricles remained normal. In the six control ears in which the round window membranes were opened and seven ears in which stapedes were mobilized or subluxated the ductus reunientes remained open with one exception of collapse in the stapes mobilization series. The positions of the membranous walls of the inner ears remained normal with the exception of collapse in the utricles. In the nine ears undergoing sacculotomy the ductus reunientes were all open. The positions of the membranous walls were normal with the exception of one which showed hydrops in the cochlea, saccule and utricle and blockage of the endolymphatic duct presumably due to surgical complication.

Fig. 5 In this specimen the ductus reunientes was obliterated and two months later the endolymphatic duct was blocked. (A) The ductus reunientes (DR) was obliterated through the round window membrane. U Utricle/H horizontal canal. (B) The cochlea shows endolymphatic hydrops. (C) The endolymphatic duct (ED) is obliterated and the saccule (SA) shows hydrops. Arrow points to the remnant of degenerated otolithic membrane attached to the distended saccular membrane. A higher magnification is shown in Fig. 6C. U Utricle. (D) The utricle (U) shows hydrops (arrows). Collapse of the horizontal canal near its ampulla (EF) is specimen preparation artifact.



In the series of 11 animals in which the endolymphatic duct was blocked two months after surgical obliteration of the ductus reuniens through the round window hydrops was observed in all cochlear saccules and in 10 utricles (Fig. 5). The magnitude of distension of these membranes was similar to that which follows blockage of the endolymphatic duct alone. In 8 of 11 ears the distended walls of the saccules contained small remnants of the atrophic otolithic membranes, structures similar to those observed on the collapsed saccular maculae in the series undergoing blockage of the ductus reuniens (Fig. 6). Other histopathological changes were neurosensory lesions at the basal ends or in both basal and apical turns similar to those seen in the ductus reuniens series. Two specimens showed sensory cell atrophy in small areas of the saccular maculae. The sensory cells of the utricular maculae and cristae were normal except for three posterior cristae which showed degeneration.

### DISCUSSION

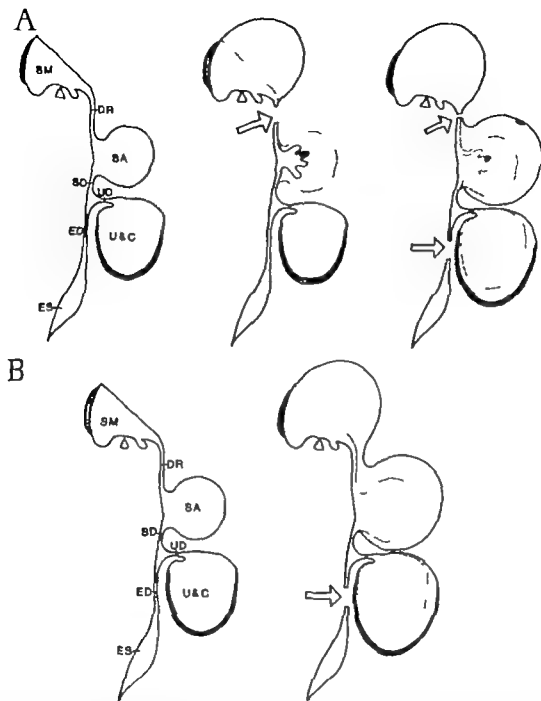
It might be argued that all these pathological alterations could be the result of a non-bacterial traumatic labyrinthitis and therefore are not related to obliteration of the ductus reuniens. Of course all invasive surgical procedures produce inflammatory and reparative responses. One method of experimental control is to perform another invasive procedure which is similar but selectively different from the one under study. When such controls were performed (opening of the round window perforation of the scala media, stapes mobilization) the resultant histopathological features

were significantly different from those following obliteration of the ductus reuniens. Even in our simple sacculotomy series (Kimura et al 1977) results were unlike those of the present ductus reuniens series. Thus histopathological observations in the majority of the specimens appear to be related to obliteration of the ductus reuniens. Various other histopathological features in a small number of specimens could be attributed to labyrinthitis, specimen preparation artifact, tears in other parts of the membranous wall or simply non-healing of the fistulized membranes.

The main histological characteristic after obliteration of the ductus reuniens was the combination of cochlear hydrops, saccular collapse and normal utricle. The combination with a much lower incidence was cochlear hydrops and normal saccule and utricle. The cochlear hydrops may be explained if we presume that endolymph normally flows toward the ductus reuniens saccule and endolymphatic sac. If the ductus reuniens were blocked and endolymph were still being produced then Reissner's membrane would distend. Supporting evidence from another experiment is the demonstration of cochlear hydrops after blockage of the endolymphatic duct, as stated earlier. In a labyrinthotomy experiment in which the hook region of the cochlea was surgically destroyed the remaining parts of the cochlea developed hydrops (Hoshino & Paparella, 1970). Gussen (1978) reported that atrophy of the human macula sacculi resulted in accumulation of otoliths or otolithic membrane debris in the ductus reuniens and/or in the cochlear duct. The tissue reaction at these areas caused development of hydrops in the basal turn and even throughout the cochlea.

The saccule in the present study collapsed possibly because it was deprived of its main source of endolymph from the scala media. There are no secretory cells (dark cells) in the saccule (Kimura, 1969). A close physiological relationship between the cochlea and saccule was also shown by the collapsed Reissner's

Fig. 6. The saccular membranes of specimens in which the ductus reuniens was obliterated first followed by blockage of the endolymphatic duct two months later. (A) The saccular membrane with the remnant of the otolithic membrane (OT) is partly lifted in the hydropic saccule (SA). ST: Stapes footplate. (B) The remnant of the otolithic membrane is attached to the distended saccular membrane. The same specimen as shown in Fig. 5C. EN: Endolymph side. (C) Another specimen showing the lifted degenerated otolithic membrane attached to the distended saccular membrane. EN: Endolymph side.



**Fig. 7** Drawings summarizing present and past experimental observations. Drawings on the left side in both figures represent the normal positions of the membranes: SM, scala media; DR, ductus reuniens; SA, sacculus; SD, saccular duct; UD, utricle and canals; ED, endolymphatic duct; ES, endolymphatic sac. The cross-shaded areas represent the stria vascularis and dark cells. Note absence of dark cells in the sacculus. (A) Changes after obliteration of the ductus reuniens show

cochlear hydrops, saccular collapse with degeneration of the otolithic membrane (black dot) and normal utricle (middle drawing). When the endolymphatic duct is blocked two months later the saccular membrane distends more than the original normal position and carries the remnant of the otolithic membrane with it. The utricle becomes hydropic (right drawing). (B) The position of the membranous endolymphatic walls after blockage of the endolymphatic duct only.

and saccular membranes in hereditarily deaf dogs (Lune 1948; Schuknecht et al. 1965; Mair 1976), in white mink (Sugiura & Hilding 1970) and in white cats (Bosher & Hallpike

1965; Mair 1973) though the utricular wall remained normal. When endolymph was withdrawn from the scala media, Reissner's and saccular membranes collapsed whereas the

utricle remained unaltered (Bast & Anson 1947). In the present study the collapse of the saccular wall seen in the round window approach is not caused by direct trauma of the sacculus by the needle nor is it likely to be due to failure of the severed ductus reuniens to heal. In one control specimen of the scala media perforation series the basilar membrane failed to heal but the ductus reuniens remained open and saccular and Reissner's membranes remained in normal position. The normal position of the saccular membrane in the presence of cochlear hydrops in a small number of specimens may suggest either that endolymph may also come from the utricle or it may come from the scala media as a result of incomplete closure of the ductus reuniens. Even in the genetically involved inner ears previously mentioned the sacculles close to the side of the utricles and endolymphatic sacs were open. The utricles remained essentially normal since the mechanism for fluid secretion and absorption was not altered. Some utricular collapses could be due to specimen preparation artifact since similar conditions are occasionally seen in normal specimens.

When obliteration of the ductus reuniens was followed by blockage of the endolymphatic duct two months later all cochleae, sacculles and utricles (except one) showed endolymphatic hydrops. Cochlear hydrops may have been caused by either obliteration of the ductus reuniens or blockage of the endolymphatic duct if the ductus reuniens were not obliterated earlier. The saccular hydrops may be explained either by endolymph coming from the scala media following blockage of the endolymphatic duct in situations when the ductus reuniens was not obliterated or by endolymph coming from the utricle via the utriculo-endolymphatic valve, utricular duct and saccular duct when the ductus reuniens was obliterated. The second possibility is very much in evidence in that the degenerated otolithic membrane was observed attached to the distended saccular membrane. As previously shown, when the ductus reuniens is

obliterated the saccular membrane collapses. In the collapsed stage the membrane on top of the macula surrounds the degenerated otolithic membrane but it is evidently not firmly attached to the macula. When the endolymphatic duct is blocked the collapsed membrane with the otolithic remnant is raised and keeps stretching beyond the normal position due to accumulation of endolymph which presumably comes from the utricular side (Fig. 7). Perhaps this evidence may also serve as indirect support for the concept of endolymph drainage from the utricle and canal organs toward the endolymphatic sac as postulated by Guild (1977). It is at odds with the opinion expressed by Seymour (1959) that the fluid from the endolymphatic sac serves to insure complete filling of the utricle and canal endolymphatic spaces.

The present experiment upholds the theory of longitudinal flow of endolymph from the cochlea, sacculus, utricle and canals toward the endolymphatic sac. In spite of the fact that the radial flow theory is not supported by this study it does not mean of course that such a flow could not also be present. Also there is no proof that endolymph is solely absorbed at the endolymphatic sac. Particles injected into the perilymphatic space appear in the scala media and eventually in the stria vascularis (Hinojosa 1972) or in the outer sulcus cells and spiral prominence cells (Altmann & Waltner 1947) and a dye injected into the canal endolymph is absorbed by the dark cells (Dohlman 1964). The localized disturbance in fluid metabolism or changes in membrane characteristics could cause a localized hydrops or collapse as shown in the present study. Although this interpretation is plausible at present there is no strong or direct experimental evidence to support this concept.

The demonstration of endolymphatic hydrops limited to the cochlea following obstruction of the ductus reuniens offers a pathophysiological explanation for the auditory type of Meniere's disease. While most patients with Meniere's disease (idiopathic endolymphatic

hydrops) exhibit both hearing loss and vertigo a small number show only hearing loss. The features of the hearing loss are distinctive and are characterized by fluctuations in thresholds and dominant low tone loss early in the disease. Lindsay & von Schultness (1958) have reported the only human temporal bone study in which endolymphatic hydrops was limited to the cochlea. Unfortunately they made no comment as to the condition of the ductus reuniens in this case.

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### ZUSAMMENFASSUNG

Der Ductus reuniens wurde in 52 Meerschweinchen erfolgreich obliteriert. Die histopathologische Untersuchung zeigte, daß die Majorität der Präparate cochlearen Hydrops, Kollaps des Sacculus und einen normalen Utriculus aufwies. Diese Resultate unterstützen die Theorie eines longitudinalen Endolymphflusses von der Cochlea zum Sacculus endolymphaticus via Ductus reuniens und Sacculus. Eine Hauptquelle von Endolymph scheint die Scala media zu sein. In 11 Meerschweinchen, bei denen der Ductus reuniens unterbrochen wurde und erst zwei Monate später der Ductus endolymphaticus blockiert wurde, wurde endolymphatischer Hydrops in allen Cochleae, Sacculi und Utriculi (außer einem) demonstriert. Die Evidenz läßt vermuten, daß Hydrops der Cochlea durch Obliteration des Ductus reuniens, während Hydrops des Sacculus und Utriculus durch Blockieren des Ductus endolymphaticus verursacht werden. Reste der Otolithen-Membran, die an der gedehnten Membran des Sacculus haften, zeigten, daß die Membran des Sacculus die über der Macula kollabiert war nach Obliteration des Ductus reuniens gedehnt worden war. Dieses Experiment unterstützt das Konzept eines Endolymphflusses vom Utriculus und den Kanälen zum Sacculus endolymphaticus. Ein blockierter Ductus reuniens könnte pathophysiologisch die auditorische Form des Morbus Meniere erklären.

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## DISCUSSION

Wersäll to Klemm: I have always admired Dr Klemm's experimental surgical ability. I want to congratulate you on the quality and consistency of your studies. I find your present work very important for the understanding of endolymph circulation. I have only one question. We know now that the dark cells are only found in the areas in the vestibular part of the labyrinth where we have endolymph production. Can you speculate on the function of the dark cells?

Sjöström to Klemm: What is the rate or rapidity of endolymph flow toward the endolymphatic sac? The second question is what is the surgical approach to the ductus reuniens through the vestibule?

Soh to Klemm: Your excellent experiments leave little doubt that there is an endolymphatic flow from the cochlea to the endolymphatic duct and sac, and that blocking of this pathway results in deafness (hydrops) proximal to the obstruction. However as surgeons I must ask myself as to the functional meaning of the endolymphatic duct (or sac) obstruction. Because I (like other ear surgeons) have seen and published cases where slow bone resorption associated with deep cholesteatoma has destroyed the entire labyrinth—at times not sparing the vestibule wall (sparing only the cochlea), while the hearing before as after the operation showed normal or near-normal bone conduction. In these ears the endolymphatic duct is obviously included in the destructive process. If such ears developed histological endolymphatic distention—the so-called

"hydrops"—then this hydrops caused them little harm. This tallies well with the observation that such endolymphatic distention as found in Meniere patients is also found in multitude of other ear diseases or indeed at times in ears with no history of deafness or vertigo.

It would therefore seem that narrowing or closing of the endolymphatic duct and sac might be less dangerous than is usually assumed—and that the histological picture of a dilated endolymphatic system is unspecific and possibly does not signify a pathological process.

The conclusions of the above to the various treatments applied to patients suffering from vertigo are self-evident.

Thälmann to Klemm: I am particularly impressed by the collapse of the saccule since this is further proof of the secretory role of the dark cells which according to your previous studies are lacking in the saccule. Also the resting potential of the saccule has been shown to be merely remote manifestation of the endocochlear potential. Your experimental pathological results agree heartily with current electrophysiological concepts about the role of the saccule.

## Klemm (Reply):

T. Wersäll: I am very grateful for your nice compliment. At present I cannot give you more information on the function of dark cells. I am sure that future study will reveal more specific function of the dark cells.

T. Sjöström: It is my impression that the size of the endolymph flow toward the endolymphatic sac is rather small and slow. This opinion is based on my experience of opening the normal endolymphatic sac. However it should be noted that hydrops develops in the cochlea and saccule within 24 hours after obliteration of the endolymphatic duct. The surgical approach to the ductus reuniens through the vestibule is to open the bony wall posterior to the round window. This is accomplished by thinning the bone with a burr and deep circular groove is made with a pointed instrument. The bony disc and fragments are lifted out with a fine hook. A long curved needle is inserted to cut the ductus reuniens of which the location was predetermined by the previous anatomical study.

T. Soh: I do not have an answer to your clinical observations. The histopathological study related to the present investigation is case of hydrops limited to the cochlea without having the typical symptoms of Meniere disease as reported by Lindsay and von Schatzl (1958). Gussen reported last year that cochlear hydrops was noted in the cases of cochlear and saccular degeneration. She attributed hydrops to the degenerated otolithic membrane entering into the ductus reuniens and the saccus vestibularis where fibrosis and inflammatory reaction developed.

T. Thälmann: Collapse of the saccule membrane is a real phenomenon. I am still convinced that there are no dark cells in the saccule. The main source of endolymph is probably the scala media. Distribution of dark cells in the vestibular labyrinth is mapped in my previous publication. They are shown in all three ampullae and utricle. A part of endolymph in the saccule may come from dark cells of the utricle and ampullae.





## ZUM COGAN SYNDROM

G Zechner

*Aus der HNO-Abteilung des städtischen Krankenhauses Wien-Lainz, Österreich*

**Abstract** Cogan's Syndrome is the clinical entity of non syphilitic interstitial keratitis with vestibulocauditory symptoms. Although the disease is not rare the pathohistological findings in temporal bones are only reported twice. Our communication deals with observations made on a temporal bone out of O. Mayer's collection. The most striking findings are the endolymphatic hydrops, the degeneration of the organ of Corti, the pathology of the ductus reuniens, the fibrosis within the cisterna perilymphatica, the destruction of the maculae and the most uncommon ossification within the scala vestibuli of the basal turn. The vessels seem to show signs of angitis.

Das Cogan Syndrom wurde erstmals 1945 als nichtsyphilitisch interstitielle Keratitis mit audiovestibulären Symptomen vergesellschaftet beschrieben. Seither wurde über etwa 60 Fälle dieser Erkrankung unklarer Ätiologie berichtet, zuletzt 1976 durch Edström und Vahlne. Allerdings gibt es nur von zwei Patienten pathohistologische Befunde von Felsenbeinen: einmal 1961 von Fisher und Hellström und 1965 von Wolff, Bernhard e a. veröffentlicht. Aus der Sammlung O. Mayers war es mir möglich die Schnittserie eines Felsenbeines zu untersuchen, die die Kriterien der perilymphatischen Fibrose zeigen. Auffallend war, daß O. Mayer den Befund als sehr bemerkenswert eingestuft hatte, ihn aber nicht ausgedeutet hatte. Der Patient ist 1921 an allgemeiner Arteriosklerose gestorben und war 54 Jahre alt. Der Befund wurde von Gimplin ger verwendet, als er 1926 eine Abhandlung über Knochenneubildung im Labyrinth veröffentlicht hatte.

**Aus der Anamnese** Die Angaben sind leider spärlich. Die Eltern waren normalhörig. Ihnen war an ihrem Kind bereits in jungen Jahren das schlechte Gehör aufgefallen, angeblich aufgetreten nach einer Typhusinfektion und

einem Erysipel. Durch zunehmende Verschlechterung galt der Patient im Erwachsenenalter als "taubstumm". Klinisch bestand links ein Restgehör, rechts keine Hörempfindung, das rechte Labyrinth war katatonisch schwach, das linke normal erregbar, jedoch kein Spontannystagmus. In Folge Fehlens der Krankengeschichte und des Obduktionsberichtes kann nur noch festgelegt werden, daß der Vorstorbene nicht an Syphilis gelitten hat, allerdings war eine Schwachsichtigkeit bekannt. Auch fehlt jeder Hinweis auf eine Mittelohrentzündung oder Hornhautentzündung. Als Todesursache ist im Sterbeprotokoll unverselle Arteriosklerose eingetragen.

Eigene Befunde und Vergleich mit den Beobachtungen von Fisher und Hellström sowie Wolff und Mitarbeiter:

1. **Trommelfell** etwas verdickt durch starke Vascularisation, die Faserstruktur etwas undeutlich, jedoch keinerlei Entzündungsresiduen. Keine Perforation. Im äußeren Gehörgang soweit erhalten. Haut und Anhangsdrüsen unauffällig wie auch bei Fischer und Hellström.

2. **Mittelohr**: Im Lumen kein pathologisches Sekret, zarte Schleimbaut ohne Entzündung oder Narben. Die ovale Fenster nische frei, ohne Sekretretention. Der Steigbügel samt Muskel unauffällig. Der Kanal zum runden Fenster frei, die Membran zart. Wolff u a. beschreiben neugebildeten Knochen

Herrn Univ. Prof. Dr. E. H. Mayer zum 70. Geburtstag in Dankbarkeit gewidmet.  
Mit Unterstützung des Fonds zur Förderung der wissenschaftlichen Forschung in Österreich.



Abb 1 HE gefärbt, Gefäßbündel entlang des Nervus facialis

innerhalb der verdickten Membran. Um den Gesichtsnerv gelagerte Gefäße am besten zu sehen nahe der ovalen Fenstergegend zeigen Veränderungen. Die Venen sind sehr weit und teilweise thrombosiert, die Arterien mit verdickter Wand haben eine homogenisierte Media und herdförmig verquollene Intima (Abb 1).

3 Schnecke. Wie im gesamten häutigen Labyrinth finden sich reichlich Pigmentablagerungen in den bindegewebigen Anteilen der Kanalwände. Am auffälligsten ist die starke Ausweitung des Ductus cochlearis in allen drei

Windungen am stärksten in der Gegend des Helicotrema, durch welches eine richtige Herniation erfolgt. In der Basalwindung liegt in der Scala vestibuli lockeres areoläres Bindegewebe. In dieses eingebettet hyaline Schollen und der Labyrinthkapsel anliegend Herde neugebildeten Knochens (Abb 2). In der Mittel und Spitzenwindung hingegen ist die Scala vestibuli aufgebraucht, die Reißnersche Membran liegt praktisch dem Modiolus an. Eine hernienartige Ausstülpung der zerdehnten Reißnerschen Membran in die mit Bindegewebe aufgefüllte Scala vestibuli ist



Abb 2 HE gefärbt, Basalwindung der Cochlea mit fibrose Knochenausbildung in der Scala vestibuli

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Unsere Befunde nach ist sie ein Teil der herdförmigen perilymphatischen Fibrosierung die beherrschendes morphologisches Substrat dieser Erkrankung ist

4 Die Veränderungen an der Stria vascularis möchten wir nicht wie Wolff und Mitarbeiter als Hauptursache des Hydrops anführen. Sie könnten sicher beitragen durch vermehrte Lymphproduktion was aber bis heute nicht beweisbar ist

Unsere morphologischen Befunde erlauben aber auch Rückschlüsse auf die Ätiologie der Erkrankung. Da unser Fall allerdings kein akuter war können wir zu den sehr bemerkenswerten Befunden erhoben von Edström und Vahlne nicht Stellung nehmen. Wenn wir allerdings die Beobachtungen an den Gefäßen im Felsenbein als panarteritis-nodosa ähnlich festlegen so wird die Störung im Immunsystem, über welche sie als Infektionsfolge berichten sehr wichtig. Auch unser Patient erlitt den ersten Schub des Hörverlustes im Rahmen einer Infektion. Ob und wie eine Autoimmunerkrankung daraus entstanden ist können wir mangels Befunden an anderen Organen nicht festlegen. Aber die Veränderungen an den Gefäßen rund um den Nervus facialis und an der Arteria labyrinthi sowie die Thrombosen in den Venen der Bogenkanalwand und der Begleitvene des Aqueductus vestibuli können als pathohistologischer Beweis gelten. Wir möchten deshalb Oliver und Mitarbeiter sowie Fisher und Hellström unterstützen und auch Cogan und Dickerson, die annehmen daß das Cogan Syndrom eine Teilform der Panarteritis nodosa ist, ähnlich der Wegener'schen Granulomatose in unserem Fachgebiet. Somit wäre das Syndrom den Autoimmunerkrankungen zuzurechnen, wofür auch die Therapieerfolge mit hochdosiertem Cortison berichtet von Smith 1970 sprechen würden.

## ZUSAMMENFASSUNG

Es wird über pathohistologische Befunde erhoben an einem menschlichen Felsenbein beim Cogan Syndrom berichtet. Die beherrschendsten Beobachtungen sind: 1

Fibrose im perilymphatischen Raum Scala vestibuli cysterna perilymphatica vestibuli rund um den oberen Bogenkanal und den Ductus rotundus. 2 Ossifikationen im perilymphatisch-fibrosierten Annulus Scala vestibuli der basalen Schneckenwindung, Vestibulum. 3 Zerstörung der Stützendothelien: Cortisches Organ macula sacculi und utriculi. 4 Endolymphatischer Hydrops mittlere, obere Schneckenwindung, Sacculus, Utriculus oberer Bogenkanal. 5 Perisacculäre Fibrose im Ductus endolymphaticus und Blockade der Basal'schen Klappe. 6 Panarteritis nodosa ähnliche Gefäßveränderungen an der Arteria labyrinthi den Venen von den Aqueductus vestibuli und an allen Gefäßen aus dem Nervus facialis. Auf Grund dieser Veränderungen nehmen wir an daß es sich beim Cogan Syndrom um eine lokalisierte Erkrankung aus dem Formenkreis der Autoimmunerkrankungen handelt.

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## DISCUSSION

Kellemen to Zechner: Records of birth of Cogan syndrome at the Massachusetts Eye & Ear Infirmary until its knowledge grew as shown in the material presentation.

u. a. erwähnt. Auch konnten wir keine blauen Mäntel in der Labyrinthkapsel erkennen.

5 Der *Aquaeductus vestibuli* zeigt eine Fülle von Veränderungen. Die Bastische Klappe am Eingang ist durch perilymphatische Bindegewebswucherungen verzogen, kaum erkennbar. Das perisacculäre Gewebe ist stark fibrotisch, das Lumen eingeengt, dort wo es halten mit Kolloidkügelchen ausgefüllt.

Das duranahende Ende ist extrem schlitzförmig. Die Befunde an den Begleitgefäßen erscheinen uns sehr wichtig. Die Vene ist thrombosiert und die Arterien um die *Pars rugosa* fehlen fast vollkommen. Dies leitet über zur *Arteria labyrinthi*, welche wir im *Porus acusticus internus* mit stark verdickter Wand sklerotischen Plaques und deutlicher Intimaverquellung beobachten konnten. Eine entzündliche Reaktion perineural haben wir nicht gesehen. Spärlich zellige Infiltrationen scheinen nur perivascular zu liegen.

*Besprechung der Befunde.* Die erhobenen Befunde sprechen für eine perilymphatische Fibrose, wie sie kennzeichnend ist für das Cogan-Syndrom. Neben der Fibrose besteht herdförmige Knochenbildung in der *Scala vestibuli* und in der perilymphatischen Cyste des Vorhofs. Die Ektasien des Endolymphschlauches fanden wir am stärksten ausgeprägt im *Ductus cochlearis* der mittleren und oberen Schneckenwindung, im *Sacculus* mehr als im *Utriculus*, aber auch im oberen Bogen gang. Daraus glauben wir zwei Dinge ableiten zu können:

1 Die Erkrankung befällt das Innenohr herdförmig.

2 Die Gefäße scheinen eine wichtige Rolle zu spielen, was ja auch schon Fisher und Hellström, Cody und Williams u. a. mehr angenommen haben.

Die von uns erhobenen Gefäßbefunde haben einerseits große Ähnlichkeit mit paratinitis nodosa ähnlichen Veränderungen, aber auch starker Arteriosklerose. Laut Obduktionsprotokoll ist dies auch die Todesursache des Patienten.

Die Erkrankung muß in Schüben verlaufen.

Alls morphologische Belege können wir anführen:

1 Die hernienartige Ausbuchtung der Reissner'schen Membran in die von Bindegewebe bereits ausgefüllte *Scala vestibuli* der basalen Schneckenwindung.

2 Der unregelmäßig zerdehnliche *Sacculus* - *Utriculus* und Bogengangsapparat allerdings anders als beim typischen Endolymphhydrops. Dies rückt eine Annahme Arslans in ein anderes Licht. Bei der Ausweitung des Endolymphschlauches spielt der Perilymphraum nicht nur eine passive Rolle. In unserem Falle scheint das perilymphatische Bindegewebe einen deutlichen Zug auf die Wände des häutigen Labyrinths auszuüben.

Vom sogenannten idiopathischen Endolymphhydrops grenzt sich die Erkrankung durch folgende morphologische Details ab:

1 Die Sinnesendstellen sind insbesondere in der Schnecke stark pathologisch verändert.

2 Die *Stria vascularis* zeigt neben Colloidzysten Kalkeinlagerung, auch gefäßbezogene Befunde.

3 Knochenneubildungen im Perilymphraum, was auch bereits Wolff und Mitarbeiter ausgeführt haben.

Der Endolymphhydrops ist demnach Folge der Grundkrankheit. Dazu möchten wir folgende morphologische Beiträge geben:

1 Die perilymphatische Fibrose scheint die locale Endolymph-Perilymphpassage stark zu behindern. Der Hydrops ist nach Jahren mitbedingt durch eine Störung der Perilymph-Endolymphschanke.

2 Die massiven Veränderungen um den *Ductus reuniens* werfen aber die immer wieder diskutierte Frage nach der longitudinalen Endolymphcirculation auf. Eine Stenose, Verziehung an dieser Engstelle kann Ursache sein für den starken Hydrops in der Cochlea, so wie wir Verziehungen oder Blockade des häutigen Anfangsteiles des *Ductus endolymphaticus* als Ursache für den Hydrops an den Strukturen des Vorhofs angegeben haben.

3 Die perisacculäre Fibrose muß Einfluß haben auf die Resorption von Endolymph.

## SENSITIVE PERIOD TO ACOUSTIC TRAUMA IN THE RAT PUP COCHLEA

### *Histological Findings*

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**Abstract** In previous paper we demonstrated physiologically sensitive period for acoustic trauma in the rat pup cochlea, with maximum at 22 days of age. Seven or 60 days after noise exposure these 22-day exposed rats were used for light and electron microscopy. At 7 days, surface preparation revealed restricted damage to the basal cochlear coil, while with electron microscopy signs of cytoplasmic degeneration appeared in the great majority of basal coil structures. Ten months after noise exposure these structures have completely degenerated. These results were discussed in terms of mechanical versus metabolic explanations of the critical period for acoustic trauma.

the sensitive period to acoustic trauma. Light and electron microscopic data give a partial answer to this question.

### METHODS

Six male white rats (Sprague Dawley) exposed at 22 days of age to a 170 dB SPL white noise and showing strong PTSs (60-80 dB between 6 and 20 kHz, for details see Lenoir et al 1979) were investigated. Histological preparations were made either 7 days (3 rats) or 7 months (3 rats) after traumatic exposure. Animals were anaesthetized with an i.p. injection of Pentotal. The cochleae were quickly removed and then immersed in 1% osmium tetroxide solution for 1 h. After washing and dehydrating they were embedded in Spurr resin. Three types of histological techniques were used to study the cochlear surface preparations (Spoendlin & Brun, 1973) giving an idea of cochleograms: semi-thin transverse sections, also checked by light microscopy and ultra-thin sections mounted on Formvar coated grids and examined with a JEOL 100C electron microscope.

### RESULTS

#### 1 Seven days after traumatic exposure

##### 1.1 Surface preparations. Only the basal coil of the cochlea showed slight damage other

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A supra-normal period of sensitivity to acoustic trauma during development (discussed in a recent review by Saunders & Bock 1978) has been physiologically demonstrated in kittens (Price 1976) and hamsters (Bock & Saunders, 1977). It has also been histologically suggested in guinea pigs (Falk et al 1974) and hypothesized in human babies (Douxé et al 1976). We investigated rats and found (Lenoir et al 1979a) that when exposed to 120 dB white noise lasting 30 minutes rats 16 to 40 days of age showed strong permanent threshold shifts (PTS). PTSs were maximal for high frequency tones when animals were exposed at day 22. The finding of maximal sensitivity correlates well with data on rat cochlear maturation (Wada 1923; Crowley & Hepp-Raymond 1966; Lenoir et al 1980b) which appears to end at about 20 days of age.

The present study complements our physiological findings (Lenoir et al 1979). The purpose was to try to determine what kind of structures in the cochlea are responsible for

*Subotić to Zechner:* Do you think that the connective tissue in the perilymphatic sphere is caused by the changes in labyrinthine circulation or by infection. I agree with you that the blockade of the endolymphatic duct in human temporal bones as in congenital malformations like Mondrini or in cases with temporal bone fracture causes a hydrops which proves that the congenital flow does exist.

*Zechner (Reply)*

*To Kelemen:* Thank you very much for your kind comments. In Cogan's syndrome we had first-hand information from the Massachusetts Eye and Ear Infirmary

*To Hui, Ing:* The reason for death in the pathology report was general arteriosclerosis within all areas of the body. Cortisone treatment needs a high initial dosage but then we substitute partially with Endocort<sup>®</sup> and at reasonable dosage we administer Immurex<sup>®</sup> instead of Endocort<sup>®</sup>.

*To Subotić:* We saw no signs of labyrinthitis and de bone formation we calculate as ossification within the perilymphatic space after a special vascular disease. The best sample of blockage is your paper on Mondrini malformation.



Figs 3-11 EM aspects of damaged structures in the basal coil of pup cochlea 7 days after traumatic exposure.

Figs 3-4 Apical pole of OHCs. Note numerous lysosomes (L), vacuoles (V) and Golgi structures (G). 16000.

Figs 5-6 IHC showing the same cytoplasmic inclusions as in OHC. Note on Fig. 5 damaged stereocilia (arrow). 10000 (5), 17000 (6).

Figs 7-8 Afferent fibres spiralling between Dester' cells with vacuoles and myelin figures (MF). 16000 (7), 9000 (8).

Figs 9-10 Efferent endings in OHC. Note the abnormally low density of synaptic vesicles and myelin figures (MF). 15000 (9), 16000 (10).

Fig 11 Myelin figure complex (Sponadun 1970) in supporting IHC. 13000.





Fig 1 Surface preparation in the basal coil of rat pup cochlea 7 days after traumatic exposure. Only very few small holes as the one marked by arrow were seen at OHC level. Nomarski optics  $\times 50$ .



Fig 2 Cross section made through the damaged area seen on fig 1. OHCs are missing and supporting cells hypertrophied. Nomarski optics  $\times 50$ .

coils looked normal with this technique. In 3 or 4 areas small holes were noticed (Fig 1) each of which consisted of 1 to 8 missing outer hair cells (OHCs). Generally the corresponding external pillars were either missing or damaged. Missing OHCs were most often seen in the first row.

**1.2 Semi-thin sections** Transverse sections made on the localized damaged areas (see 1.1) confirmed the absence of OHCs and the damage to external pillars which appeared distorted or broken (Fig 2). Epithelial cells of the internal spiral sulcus and Hensen's cells were hypertrophied. In neighbouring areas the cochlea looked normal in light microscopy except again for the hypertrophy of supporting cells.

**1.3 Electron microscopic observations** Regardless of the place of transverse ultra-thin sectioning within the basal coil of the cochlea sensory and even neural structures presented signs of degeneration which of course were unnoticeable with light microscopy. The great majority of OHCs were distorted and numerous cytoplasmic modifications appeared. The apical pole of the cell just underneath the cuticular plate contained vacuoles, lysosomes and dark mitochondria (Figs 3, 4). Inner hair cells (IHCs) did not show any gross morphological changes but again cytoplasmic signs of metabolic impairments appeared similar to but less numerous than those seen in OHCs. In addition IHCs showed an increase

in endoplasmic reticulum forming Golgi-like apparatus and abnormal stereocilia (Figs 5, 6).

Abnormalities were also found in neural endings inside the organ of Corti. The majority of afferents below the OHCs spralling between Deiter's cells showed vacuoles and myelin figures (Figs 7, 8). Efferent endings to the OHCs also contained vacuoles and myelin figures. The density of vesicles was abnormally low (Figs 9, 10). At the level of IHCs the neural supply appeared normal.

Cytoplasmic degenerating processes were also encountered in supporting cells (Fig. 11). The inner spiral sulcus cells showed besides the hypertrophy already mentioned cytoplasmic evaginations in the cochlear duct. In the external sulcus Hensen's cells and Claudius cells were also hypertrophied.

## 2 Two months after traumatic exposure

**2.1 Surface preparation** Damage had now extended and about 80% of basal OHCs were missing; the remaining 20% looked greatly disorganized. No apparent lesions were seen at the IHC level.

**2.2 Electron microscopic observations** Sensory and neural structures showed pronounced degeneration. At locations where OHCs were present in surface preparations there remained only the cuticular plate with abnormal hairs and a small cytoplasmic portion. IHCs now also appeared degenerated.

the fact that Nuel's spaces which have just differentiated around day 15 place OHCs and their nerve endings directly in contact with a corticolymph possibly poisoned by endolymph. In contrast, IHCs are much more protected throughout their life by neighbouring supporting cells.

Lastly vascular disorders (Lawrence 1970; Hawkins 1971) cannot be excluded when explaining the sensitive period to trauma. Again a strong correlation exists between that period and the time when cochlear vessels (spiral and strial ones) seem to switch from immature to mature stage (Wada 1923; Lenoir et al 1980). The characteristic decrease of vascularization may coincide with a peculiar weakness of sensory structures not yet metabolically stabilized.

To account for the supra-normal sensitivity to acoustic trauma at 170 dB SPL, these various hypotheses probably need to be considered together. Experiments are now under way to try to determine the sensitive period more precisely by lowering and filtering the traumatic noise. This is hopefully one way to discriminate among histological impairments which occur first and are responsible for immediate deafness and those which are secondary consequences and extend the apparent traumatized areas.

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## RÉSUMÉ

Dans un article précédent, nous avons montré que la cochlée du raton présente un maximum de sensibilité au trauma acoustique au 22<sup>e</sup> jour postnatal. Sept et 60 jours après l'exposition au trauma des investigations histologiques ont été réalisées en microscopie optique et électronique. Sept jours après le trauma, les préparations de surface ne révélant que des dégâts limités, alors qu'en microscopie électronique des signes de dégénérescence cytoplasmique apparaissent dans toutes les structures de tout bord de la cochlée. Ces résultats sont discutés en fonction des explications mécaniques ou métaboliques de trauma durant la période critique.

## ZUSAMMENFASSUNG

In einem vorübergehenden Artikel haben wir gezeigt, daß die Cochlea der Jungtiere am 22. Tage nach der Geburt eine Hörschwäche gegenüber Hörschaden aufweist. Am 7 und 60 Tage nach dem Trauma des Tralles ausgesetzt wurden, wurden histologische Untersuchungen mit dem optischen und elektronischen Mikroskop unternommen. Am 7 Tage nach dem Trauma zeigten Beobachtungen mit dem elektronischen Mikroskop Zeichen eines Verfalls des Zellplasmas in allen Strukturen in der Basis der Cochlea. Hier wollen wir diesen Befund in bezug auf die mechanischen und metabolischen Erscheinungen der Hörschwäche in der kritischen Periode besprechen.

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Nerve fibres had completely disappeared at the OHC level with Deiter's cells filling the space left free by the degenerated sensory cells. Below IHCs degeneration had occurred in the nerve supply and the retrograde process affected most of the laminar fibres.

## DISCUSSION

Apart from the mechanical lesions seen immediately after exposure to very intense sounds (around 130 dB SPL, see Spoendlin & Brun 1973) the majority of traumatic processes in the cochlea originate from metabolic impairments. According to Bohne (1976) three main factors may contribute to metabolic trauma: enzymatic depletion within the sensory cells due to overfunctioning; endolymph penetrating inside the organ of Corti via small holes in the reticular lamina; and vascular disorders. This kind of trauma is characterized by a slow and progressive degeneration of sensory and nerve structures which takes several months to fully develop (Bohne 1976; Spoendlin 1976). However, electrophysiological and behavioral measures show threshold elevations immediately following noise exposure (Hawkins et al. 1976). The kinetics of the trauma found in our developmental experiment agree very well with these findings.

The main observation in our previous physiological study (Lenoir et al. 1979a) was of a period of heightened sensitivity to acoustic trauma at a time when the cochlea becomes just mature. An important question arises: can any of the above-mentioned explanations for acoustic trauma in the adult be applied to our findings of a critical period? The data reported here give a partial answer to this question.

### 1 Mechanical lesions

During the critical period a half-hour of 120 dB SPL white noise seems enough to cause minor mechanical lesions in the organ of Corti. Besides obvious damage represented by missing OHCs or external pillars, there can be more

discrete lesions on the reticular lamina which may be of major importance in the subsequent cochlear degeneration. Actually, endolymph penetration into the organ of Corti seems probable.

### 2 Metabolic impairments

The first impression which arises from our electron microscopic and physiological data is of a kind of severe metabolic exhaustion of sensory cells in such a manner that recovery is impossible. Cytoplasmic anomalies such as lysosomes, myelin figures and vacuoles have been described in adult preparations as indicating an overfunctioning of hair cells (Engström & Ades 1960; Spoendlin 1970; Spoendlin & Brun 1973). It seems quite logical to think about an increase of this phenomenon in cochleae which have just reached their adult stage but may then not be completely stabilized in terms of their metabolic capacities. There is also a possibility that the efferent endings which may be responsible for protecting the hair cells against an overfunctioning are not mature enough in 22-day-old rats to play the same role as they do in adults. We know that the large efferents to OHCs are among the last structures to develop in the cochlea (Kikuchi & Hilding 1965; Pujol et al. 1978; Lenoir et al. 1980). Some of the histological findings reported here inside efferent endings (myelin figures and the paucity of synaptic vesicles) suggest that the efferent system may have been activated too much for its developmental stage.

Another aspect of metabolic impairment suggested by our results is a possible poisoning of the organ of Corti by endolymph. This phenomenon originating from small mechanical lesions of the reticular lamina has been hypothesized in adult cochlea (Bohne 1974). To explain its increase during the sensitive period we have to suggest that the reticular lamina is not completely mature at that time. However, what leads us to a poisoning hypothesis is that outer structures are more damaged than inner ones. This may be due to

# INVESTIGATIONS ON THE CRITICAL PERILYMPHATIC PRESSURE VALUE CAUSING ROUND WINDOW MEMBRANE RUPTURE IN ANESTHETIZED CATS

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**Abstract** Increasing perilymphatic fluid pressure was found to be an important factor in the etiology of round window membrane rupture. The critical pressure causing membrane rupture was determined in anesthetized cats. Its value was found to be in the range 10-30 mmHg ( $0.5 \pm 0.1$  E.M. =  $23.4 \pm 1.1$ ). This value was compared with the pressure increases caused by thoracic and abdominal compression, cervical strangulation, forced Trendelenburg position, coughing and sneezing.

Although pressure and flow conditions in the perilymphatic space have been investigated from several aspects in the last couple of years one can find scarcely any reference in the literature about the critical perilymphatic pressure values causing rupture of the round window membrane.

In the present series of experiments—carried out in anesthetized cats—we have investigated this critical range of the perilymphatic pressure.

The pressure values causing rupture of the round window membrane were compared with those increased perilymphatic pressure values which could be measured in different, experimentally induced physiologic and pathophysiologic conditions in the same experimental animals.

## METHODS

The experiments were performed on 12 female cats weighing 2-3 kg and anesthetized with 40 mg/kg sodium pentobarbital (Ventural, Abbott, Ottignies Belgium) intravenously.

The trachea was cannulated with a glass

cannula, and the bullae osseae were prepared free on both sides from incisions led behind the ears. The round window was exposed by opening the bulla. In one group of the animals (6 cats) a 1.5 mm hole was opened with a dental drill to the perilymphatic space through the bony wall of the round window at some millimetres distance from the window. In another group of animals (6 cats) the perilymphatic space was opened by stapedectomy.

A 1.0 mm outer diameter metal cannula was inserted into the hole and fixed with Duracryl (Duracryl "O" self-curing resin SPOFA-Dental Prague) dental cement. The cannula was connected to a short polyethylene tube, fixed with Evans-blue stained physiological saline. The tube was connected to a three-way stopcock, the first branch of which was connected to a mercury manometer whose pressure values were recorded on a kymograph. The second branch was connected to an electric pressure transducer (P 23 AA Statham, Farmingdale, Puerto Rico, USA) in order to have a simultaneous possibility for recording low "break-through" pressure values on a polygraph (C.F. Gine Galileo R 105 h, O.T.E. Biomedica, Florence, Italy). The pressure in the perilymphatic space was raised slowly and continuously through the third branch of the stopcock with the aid of a Mouton syringe filled with Evans-blue stained physiological saline.

The experiments were carried out only in those animals in which the round window membrane was intact under the control of an electron microscope ( $\times 37$  magnification).

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## DISCUSSION

*Wersäll to Pujol* You mentioned two morphological criteria of damage. One was perforation of the organ of Corti. One was changes in the amount of lysosomes. Are your defects real holes or areas of degeneration of hair cells without holes. Have you tried to quantify the damage inside the hair cell? Did you do any scanning EM?

*Spoendlin to Pujol* The poisoning of the outer hair cells by endolymph which penetrates through holes and ruptures of the reticular lamina is certainly an interesting possibility. However, this could not explain the delayed degeneration of outer hair cells which you observe between one week and 2 months after the exposure. Localized ruptures in the reticular lamina have a tendency to be closed by a scar very rapidly following the exposure.

*Verwoerd to Pujol* What do you mean by "the last stages of cochlear maturation"? Did you attempt to repeatedly expose rats to noise during the critical period?

*Pujol (Reply)*

*To Wersäll and Spoendlin* We intend to do some scanning EM which, of course, might give us a lot of information.

The structural impairments of hair cells and nerve endings shown in our EM 7 days after noise exposure indicate that a degenerating process is under way which will destroy the organ of Corti almost completely after months. That process can be explained by the poisoning hypothesis. But these impairments do not seem strong enough to account for the immediate deafness. Then, another explanation is that they can be signs of a severe metabolic exhaustion of structures just achieving their maturation and not completely yet stabilized.

*To Verwoerd* The last stages of the maturation of the organ of Corti (which coincide with the beginning of the critical period) concern the OHC and their efferent innervation.

I can answer indirectly as we haven't done a double exposure to noise. But we have sensitized a cochlea (during the critical period) by low doses of antibiotics; the effect of noise exposure is then much more pronounced.

The slowly developing process of the membrane rupture was recorded simultaneous with the pressure measurements by means of a Minolta XG<sub>2</sub> automatic camera combined with an auto-winder.

Perilymphatic fluid pressure changes under the influence of cervical strangulation forced Trendelenburg position (Miriszalai 1977) chest and abdominal compression coughing and sneezing were tested in each experiment before measuring the critical rupture-causing pressure values.

Cervical chest and abdominal compression was produced manually. The forced Trendelenburg position was produced by lifting the hind limbs up to vertical position. Coughing and sneezing was provoked by mechanical stimulation of the trachea and the nose with a polyethylene tube. The maximum perilymphatic pressure changes measured during these model situations were compared with the critical pressure values causing round window membrane rupture.

A detailed histologic (electronmicroscopic) study of the structure of the round window membrane was published by us previously (Miriszalai et al 1978). Since the situation and the extent of the membrane damages were quite different in each of the experiments, histologic investigations were not carried out in connection with the present study.

## RESULTS

The dynamics of the round window membrane rupture are demonstrated in Figs. 1, 2, and 3 respectively. Fig. 1 shows the appearance of the normal healthy round window membrane in the cat. Fig. 2 shows how the membrane swells out from the perilymphatic space under the influence of the increased pressure caused by the Evans blue stained saline injection, the membrane is reaching the stage prior to rupture. Fig. 3 demonstrates the picture of the membrane rupture caused by an experimentally induced increase in the perilymphatic pressure.

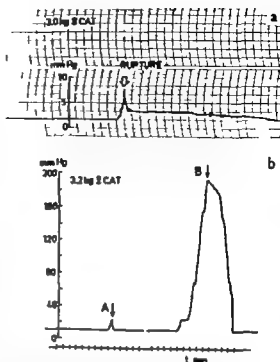


Fig. 4 Original perilymphatic pressure recordings showing the moment of the round window membrane rupture in cat ( ). (a) Electromanometric recording. (b) Recordings obtained in the two inner ears (A and B) of the same cat with mercury manometer. ↓ The moment of the rupture.

Fig. 4 demonstrates some typical original pressure recordings of the increasing perilymphatic fluid pressure and of the moment of membrane rupture. Part (a) demonstrates an electromanometric recording in which the rupture of the membrane occurred at a pressure value which can be considered as an average rupture-causing pressure in the present experiments. Part (b) shows two kymograph recordings (obtained with mercury manometer) from the two inner ears of the same animal. These recordings demonstrate the observation that the pressure value causing round window membrane rupture can be quite different in the two inner ears of the same animal.

Perilymphatic pressure values causing the rupture of the round window membrane in the experiments are summarized in Table I. As can be seen, the critical average pressure value causing rupture of the round window mem-



*Fig 1* Intact healthy round window membrane of the cat  $\times 8.5$



*Fig 2* Round window membrane under the influence of critically increased perilymphatic fluid pressure  $\times 4$



*Fig 3* Ruptured round window membrane of the cat.  $\times 8.5$

Table II *Maximum perilymphatic pressure increases (in mmHg) under the influence of test situations*

| Type of the test       | Cervical strangulation | Thoracic compression | Forced Trendelenburg position | Coughing | Sneezing |
|------------------------|------------------------|----------------------|-------------------------------|----------|----------|
| Number of exp. animals | 5 cats                 | 7 cats               | 5 cats                        | 5 cats   | 5 cats   |
| Inner ear measurements | 8 ears                 | 11 ears              | 10 ears                       | 7 ears   | 5 ears   |
|                        | 9 cases                | 24 cases             | 22 cases                      | 36 cases | 29 cases |
| $\bar{x}$              | 1.09                   | 0.75                 | 1.79                          | 1.14     | 1.18     |
| S.E.M.                 | 0.31                   | 0.11                 | 0.34                          | 0.12     | 0.15     |
| S.D. $\pm$             | 0.93                   | 0.53                 | 1.58                          | 0.72     | 0.80     |

sure changes are shown on Table II. As can be seen in this table, the most significant pressure increase was observed during the forced Trendelenburg position. In some of these cases we have observed a pressure increase of 5.8 and 4.0 mmHg magnitude, which values are close to the lowest pressure values causing round window membrane rupture in our experiments.

## DISCUSSION

The symptoms of the round window membrane rupture—according to the literature data—can be observed in the form of serious disturbances of the cochlear and vestibular function and ear noises (Simmons 1968, Goodhill 1971, 1973, Pallen 1977, Freeman et al. 1974, Healy et al. 1974, Taylor & Bricknell, 1976, Altam 1976, Althaus 1977, Goodman & Moroka, 1978, Kleinfeldt 1978, Mohrner et al. 1978, Behbehani & Kastenbauer 1978).

Experimental studies published in the last couple of years have provided a considerable amount of data on the pathophysiological background of the disturbances (Simmons et al. 1962, Arnold & Liberg 1972, Lamkin et al., 1975, Axelsson et al. 1977, Ivarsson & Pederlen, 1977, Weiskopf et al. 1978).

The data of Simmons (1962, 1968) and Goodhill (1971, 1973) have called attention to the significance of the perilymphatic pressure changes in the development of perilymphatic

fistulas. The aim of our present investigations was to prove the connection between the perilymphatic pressure increase with the rupture of the round window membrane in experimental conditions and also to determine the pressure range of the perilymphatic fluid at which round window membrane rupture can occur. The results of the animal experiments presented show that increased perilymphatic pressure can lead definitely to rupture of the round window membrane. The average of the critical pressure values was between 20 and 30 mmHg. The highest and the lowest pressure values at which membrane rupture was observed in the experiments were 66 and 6 mmHg respectively. In this pressure range there was no accompanying oval window membrane rupture. This fact makes it certain that the tissue structure of the oval window membrane is more resistant to the increased pressure than is that of the round window membrane in spite of the observation that the tissue layer covering the perilymphatic space from inside appears to be a homogenous arachnoid structure (Franke 1979).

According to our present results, the pressure values causing rupture of the round window membrane can show considerable individual differences and significant differences were found in this respect even in the two inner ears of the same animal. We assume that this phenomenon is probably due to the structural and mechanical resistance differences of the membranes. The perilymphatic pressure



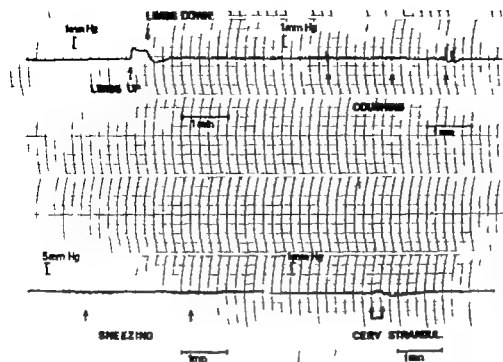


Fig 5 Original pressure recordings, showing the increased perilymphatic pressure in the cat under the influence of: (a) forced Trendelenburg position (hind limbs elevated in vertical position) (b) coughing, (c) squeezing, (d) cervical strabulation

brane in 12 ears of 8 anesthetized cats is equal to  $23.4 \pm 17.1$  mmHg ( $\bar{x} \pm \text{S.E.M. S.D.} = 4.9$  mmHg). The upper and lower limits of the critical pressure values in healthy intact membranes are 66 mmHg and 6 mmHg respectively. The rupture-causing critical pressure values can be quite different in the two ears of the same animal even in the case when both of the membranes were found healthy under the operating microscope.

Table 1 Critical perilymphatic pressure values causing round window membrane rupture in anesthetized cats

| No of exp animal | Body weight (kg) | Pressure causing rupture (mmHg) |
|------------------|------------------|---------------------------------|
| 1                | 3.5              | 18.0                            |
|                  |                  | 66.0                            |
|                  |                  | 26.0                            |
| 3                | 3.0              | 10.5                            |
|                  |                  | 22.0                            |
| 4                | 3.0              | 1.5                             |
|                  |                  | 6.0                             |
| 5                | 3.2              | 14.0                            |
| 6                | 2.3              | 22.0                            |
| 7                | 3.5              | 42.0                            |
| 8                | .8               | 34.0                            |
|                  |                  | 8.0                             |

$\bar{x} = 23.4$  mmHg S.E.M. = 17.1 mmHg S.D. = 4.9 mmHg  $n = 12$ .

We were able to perform reliable pressure measurements on both inner ears of 4 animals. The average rupture-causing pressure was 17.1 mmHg on the one side and 35.6 mmHg on the other side of the same animal—thus rupture could be produced in these cats with a 18.5 mmHg pressure difference between the two ears. The round window membrane was visibly enlarged in 4 inner ears of 4 animals (outside of the 8 healthy cats) in another series of our experiments.

Membrane rupture was observed at 110 and at 186 mmHg in two of these membranes and in the other two ears it was not possible to produce a rupture even above 230 mmHg perilymphatic pressure. In these latter two cases the microscopic observation of the enlarged membranes showed an interesting picture: the Evans blue stained saline penetrated between the layers of the round window membrane and we could observe a suffusion of this fluid below the perosteum covering the bone around the window.

An original electromanometric pressure recording in Fig 5 demonstrates the pressure changes in the perilymphatic space under the influence of the pressure increasing test manoeuvres. The average values of these pres-

## DISCUSSION

*Tonndorf to Murzin:* I wish you had divided your cases into two groups: (1) in which you raised the pressure extremely slowly so as to produce something like pure pressure effect, and (2) in which you changed the pressure fast as you apparently did in your experiments, so as to obtain the effect of acceleration. These are two different things.

*Sandoz to Tonndorf:* You are right. However, we found no differences between the critical pressure values when we have compared the effect of sudden pressure increase and pressure elevation caused by a very slow infusion pump. Another aspect of this question, in the reality in those situations we tried to modelling here/coughing, sneezing/ the pressure increases even more rapidly as in the presented series of our experiments.

increases evoked by thoracic and abdominal compression coughing sneezing and forced Trendelenburg position caused no round window membrane rupture though the highest pressure values in these situations were close to the lowest rupture-causing perilymphatic pressure values. Hence the possibility that these situations can lead to the rupture of the membrane after membrane injuries or in the case of disfunction of the tuba cannot be excluded. These results provide data for an understanding of the clinical observations that physical effort situations can sometimes lead to round window membrane rupture.

The results of our experiments are related to the fact that in the pressure increasing situations investigated by us the elevation of the pressure generally does not reach the upper limits of the tensile strength of the round window membrane. The resistance of the intact healthy membrane structure against explosive everyday pressure increases (coughing sneezing) in the inner ear seems to be generally high.

It seems very likely therefore that membrane rupture occurs only in those cases when an extreme pressure increase affects the damaged membrane of the round window.

## ACKNOWLEDGEMENT

This work was supported by NINCDS Grant (NS-10939-04) and by the Scientific Research Council Ministry of health Hungary (1-07-0301-00-1/K).

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Abb. 1 (a) Zusammengesetzter Ausschnitt aus der Stria vascularis des Falles 1) Erste Windung: P, Promontoria spiralis, E, flache Reissner'sche Membran; darunter wunderbar begrenzte Ligamentum spirale (L). Im oberen Anteil besteht noch ein massives Stria-Ödem (P) insgesamt aufgehobene Striamorphologie und Fehlen silicischer Kapillaren. 460 (b) Zweite Windung: über der gesamten Striaausbreitung schweres Ödem mit Verlust aller charakteristischer Striastrukturen. 460

den Befunden bei der experimentellen Masugi-Nephritis den Einfluß von Nierenerkrankungen auf das Innenohr zu erhalten.

#### MATERIAL UND METHODIK

Die Felsenbeine zweier Patientinnen mit Alport-Syndrom wurden in einem Falle 1,5 Stunden post mortem, im anderen Falle 45 Mi-

nuten post mortem transmantal nach Herausnahme des Trommelfelles des Steigbügels und nach Eröffnen des runden Fensters durch Perfusion mit 3,5%igem eiskühlem Glutaraldehyd perfundiert. Nach der Sektion

Für hervorragende technische Mitarbeit danke ich Fr. B. Linenkolle.

# ÜBERLEGUNGEN ZUR PATHOGENESE DES COCHLEO-RENALEN SYNDROMS<sup>1</sup>

## *Considerations on the Pathogenesis of the Cochleo-renal Syndrome*

W Arnold

*Aus der Hals-Nasen-Ohrenklinik der Universität Düsseldorf BRD*

Es ist bekannt daß bestimmte Nierenerkrankungen mit einer zunehmenden Innenohr schwerhörigkeit emhergehen. Das Paradebeispiel hierfür stellt das Alport Syndrom dar, eine familiäre Glomerulonephritis mit Innenohrschwerhörigkeit und Augenveränderungen. Es ist dabei noch nicht bewiesen, ob die genetische Determination von vorneherein eine Degeneration bestimmter Innenohranteile mit einbezieht, oder ob die Innenohrschwerhörigkeit Folge einer besonderen Form von Glomerulonephritis ist. Bei anderen Innenohrschwerhörigkeiten im Gefolge einer Glomerulonephritis wie beispielsweise bei der diabetischen Glomerulonephritis (Kimmelstiel-Wilson) mag die Innenohrschwerhörigkeit sich aus der für die diabetische Grundkrankheit bekannten Basalmembranveränderung erklären. Ferner führen besonders Medikamente, die zur Therapie von Nierenfunktionsstörungen eingesetzt werden (Furose, munde Ethacrynsäure) zu vorerst reversiblen, bei langzeitiger Anwendung irreversiblen Hörstörungen. Die Vorstellung von einer immunologisch bedingten Schwerhörigkeit (Quick 1975) begründet sich dagegen in der Beobachtung, daß Nierentransplantierte Patienten gelegentlich einen Innenohr Hörverlust aufweisen. Oda et al. (1974) untersuchten 290 Patienten mit Nierentransplantationen beziehungsweise Patienten, die unter Hämodialyse standen, und fanden bei 43 Patienten einen eindeutigen Hörverlust in unmittelbarer Abhängigkeit von der Therapie der Nierenerkrankung. Bei der Untersuchung von 16 Felsenbeinen von 8 Hämodialyse oder Nierentransplantations-Patienten

fanden sie unter den Felsenbeinen Ablagerungen im Bereich der Stria vascularis und degenerative Veränderungen der vestibulären Rezeptoren. Ähnliche Ablagerungen hatte Gacek (1971) bereits beim Alport Syndrom beschrieben. Da diese Ablagerungen auch bei anderen angeborenen oder genetisch bedingten Schwerhörigkeiten gesehen werden (Jervell-Lange Nielsen Syndrom, Scheibe-Dysplasie), kann daraus offensichtlich keine spezifische Veränderung abgeleitet werden. Vielmehr muß man bei der Betrachtung einer gemeinsamen oder voneinander abhängigen Reaktion von Nierengewebe und Innenohrgewebe entweder davon ausgehen, daß es sowohl im glomerulären Nierensystem wie auch am Innenohr (hier vorzugsweise im Bereich der Stria vascularis) bestimmte Rezeptoren, beispielsweise für Pharmaka, gibt, die nur hier oder hier besonders stark angreifen. Oder aber es muß ausschließlich im Bereich der Nierenglomerula und der Stria vascularis immunologisch voneinander abhängige Gewebskomponenten geben, die gemeinsam reagieren (Quick et al. 1973, Arnold et al. 1976, Werdauer et al. 1977).

Die licht und elektronenmikroskopische Untersuchung zweier Fälle von Alport Syndrom, bei denen die Diagnose sowohl familienanamnestisch durch Nierenbiopsien bei verschiedenen Familienangehörigen sowie durch otologische Untersuchungen gestellt worden war, kann dazu beitragen, im Vergleich mit

<sup>1</sup> Mit Unterstützung der Deutschen Forschungsgemeinschaft (Ar 170/1)

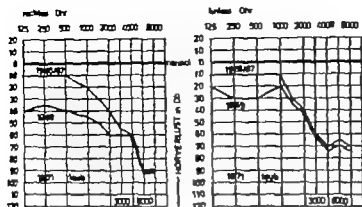


Abb. 4 Tonschwellenaudiogramme 1966, 1969 und 1971. In den letzten Lebensmonaten war die Patientin taub.

der Stria vascularis, aber nur eine geringfügige Degeneration der Prominentia spiralis. Lediglich im Bereich der dritten Windung fanden sich noch Bezirke von epithelähnlichem Aufbau mit weitgestellten Gefäßen. Der Flüssigkeitsraum des nur mehr spärlich vorhandenen Ligamentum spirale erstreckte sich bis unmittelbar unter die Epithelreste (Abb. 1a, b). Die Reissner'sche Membran war intakt, im Bereich des Limbus spiralis imponierte ein Kondensat von PAS-positivem Material an der Oberfläche der Interdentalzellen. Auch die Tectoralmembran farbte sich intensiv PAS-positiv in ihren Randbezirken an. Das Cortische Organ wies in allen Windungsanteilen einen vollständigen Verlust der inneren und äußeren Haarzellen auf, die Tunnelradialfasern fehlen in den Serienschastien (Abb. 7). Das Ganglion spirale ließ eine Degeneration der Nervenzellen erkennen, wobei die umhüllenden Mantelzellen erhalten blieben und somit häufig kochförmige Areale zu erkennen waren (Abb. 3). Tonaudiometrisch bestand bei dieser 38-jährigen Patientin zum Todeszeitpunkt eine beidseitige Taubheit (Abb. 4).

#### Fall N. 2

Bei dieser 42-jährigen Patientin mit familiärem Alport-Syndrom war mehrere Jahre vor dem Tod durch eine Nierenbiopsie durch die klinische Symptomatik einschließlich eines geringfügig zunehmenden Hochtonverlustes und einer Linsenströmung die Diagnose bestätigt

worden. Die Patientin verstarb im Zustand der kompensierten Niereninsuffizienz an einem akuten Herzinfarkt. Sie erhielt während der gesamten Erkrankungsjahre keine inneren oder toxischen Pharmaka. Die Nierenbiopsie 1974 ließ im Bereich der Nierenglomerula noch eine Verbreiterung der Basalmembranen sowie eine Verschmelzung der epithelialen Zellaußenräume erkennen, die nach David et al. (1966) und nach Zollinger & Mihatsch (1978) kennzeichnend für glomeruläre Veränderungen beim Alport-Syndrom sind (Abb. 5a, b). Zum Zeitpunkt dieser Nierenbiopsie wies die Patientin eine äußerst geringgradige, vorwiegend im Hochtonbereich gelegene Innenohrschwerhörigkeit mit deutlichem Seitenunterschied auf. In den folgenden Jahren verschlechterte sich die Nierensituation und gleichzeitig kam es von einer geringgradigen Schwerhörigkeit zu einer mittelgradigen, wiederum seitensymmetrischen Schwerhörigkeit, dabei war die stärkste Zunahme des Hörverlustes im Hochtonbereich vorhanden (Abb. 6).

Die Untersuchung der Nierenglomerula zum Todeszeitpunkt ließ eine weitgehende Degeneration und Obliteration erkennen. Elektronenmikroskopisch waren die meisten Gefäße durch eine enorme Proliferation der Epithelien und Verdickung der Basalmembranen eingeengt oder obliteriert (Abb. 7).

Die Stria vascularis war in allen Windungsanteilen voll erhalten, ausschließlich im Bereich der Reissner'schen Membran und unmittelbar



Abb 2 Cortiorgan der zweiten Windung. Degeneration der Inneren und Äußeren Haarzellen. Verlust der Stützelemente  $\times 423$

wurden die Felsenbeine entnommen so zugesägt daß die Schnecken in einem 1,5 cm großen Würfel enthalten waren und erneut über Nacht in 3,5%igem Glutaraldehyd nach fixiert. Anschließend wurden die Cochleae mit 1%igem Osmiumtetroxyd nachfixiert. Im ersten Fall wurde anschließend mit EDTA entkalkt die Schnecke in parallele Scheiben zerschnitten und eingebettet. Im zweiten Falle wurde ohne Entkalkung in hartes Epon eingebettet und der Eponblock anschließend mit einer hochtourigen Säge in millimeterdicke Scheiben zerschnitten. Die Semidünnschnitte wurden mit Methylenblau oder nach Mallory II gefärbt. Die Kontrastierung der elektronenmikroskopischen Schnitte erfolgte mit Uranylacetat und Bleicitrat. Die Untersuchung er-

folgte an einem Elektronenmikroskop Zeiss EM 9 a.

Fall Nr 1 wurde bereits 1976 publiziert (H Weidauer W Arnold *Z Laryngol Rhinol* 55 6-16 1976)

## BEFUNDE

### Fall Nr 1

Histologisch bestand hier eine diffuse Glomerulonephritis mit enormer Verdickung der Basalmembranen so daß im PAS gefärbten Präparat die Gefäßlumina weitgehend verdrängt waren. In großen Arealen waren die Glomerula vollständig obliteriert.

Das Innenohr zeigte im Bereich aller Windungsanteile eine vollständige Degeneration

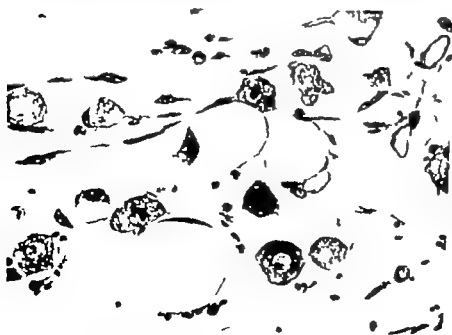


Abb 3 Ausschnitt aus dem Spiralganglion der zweiten Windung. Anzahl der Ganglienzellen deutlich reduziert. Zellen selbst pyknotisch oder verschwunden so daß keine von Mantelzellen umgebene Hohlräume resultieren. Den Ganglienzellen fehlt jegliche Myelinisation was nach Kimura für das menschliche Spiralganglion charakteristisch ist  $\times 350$

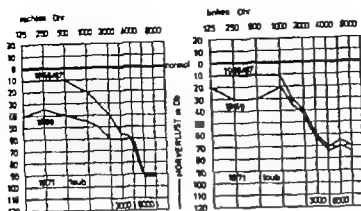


Abb. 4 Tonschwellenaushögramme 1966, 1969 und 1971. In den letzten Lebensmonaten war die Patientin taub

der *Stria vascularis*, aber nur eine geringfügige Degeneration der *Prominentia spiralis*. Lediglich im Bereich der dritten Windung fanden sich noch Bezirke von epithelähnlichem Aufbau mit weitgestellten Gefäßen. Der Filtrationsraum des nur mehr spärlich vorhandenen *Ligamentum spirale* erstreckte sich bis unmittelbar unter die Epithelreste (Abb. 1a, b). Die Reissner'sche Membran war intakt. Im Bereich des *Limbus spiralis* imponierte ein Kondensat von PAS-positivem Material an der Oberfläche der Interdentalzellen. Auch die Tectorialmembran färbte sich intensiv PAS-positiv in ihren Randbezirken an. Das Cortische Organ wies in allen Windungsanteilen einen vollständigen Verlust der inneren und äußeren Haarzellen auf. Die Tunnebradialfasern fehlten in den Serienschnitten (Abb. 7). Das Ganglion spirale ließ eine Degeneration der Nervenzellen erkennen, wobei die umhüllenden Mantelzellen erhalten blieben und somit häufig hochförmige Areale zu erkennen waren (Abb. 3). Tonaudiometrisch bestand bei dieser 38-jährigen Patientin zum Todeszeitpunkt eine beidseitige Taubheit (Abb. 4).

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Die Untersuchung der Nierenglomerula zum Todeszeitpunkt ließ eine weitgehende Degeneration und Obliteration erkennen. Elektronenmikroskopisch waren die meisten Gefäße durch eine enorme Proliferation der Epithelien und Verdickung der Basalmembranen eingengt oder obliteriert (Abb. 7).

Die *Stria vascularis* war in allen Windungsanteilen voll erhalten, ausschließlich im Bereich der Reissner'schen Membran und unmittelbar





Abb. 5(a) Glomerulumausschnitt mit zwei Schlingenlumina (L) die allseits von fusulierten teilweise breitflächig aufsetzenden Podocytenfortsätzen abgedeckt sind (P). Nierenbiopsie 2 Jahre vor dem Tode.  $\times 4500$  (b) Ausschnitt der Glomerulakapillarwand mit beidseitiger Verschmelzung plumper Podocytenfortsätze (P).  $\times 5000$

K.J. 4736 9.10.78 Alport Syndrom Diskr 100%

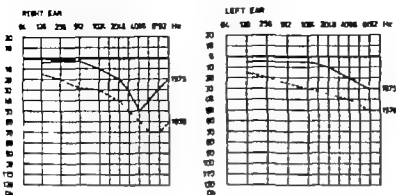


Abb 6 Tonschwellenaudiogramme 1975-1978. Die Patientin hatte bis zum Tode ein gutes Sprachverständnis.

nabe der Prominentia spiralis bestand ein breites Interzellularoedem. An der Oberfläche fanden sich Protoplasmaprotusionen und schwammige Zellaufreibungen, die als patholo-

gische Veränderungen betrachtet werden müssen (Abb 8a, b, c). In Senarschnitten waren die äußeren Haarzellen bis auf wenige Abschnitte im Bereich der Basalwindung in allen



Abb 7 Nierobiopsie zum Todeszeitpunkt. Weitgehende Degeneration und Obliteration der Glomeruli, fehlende Gefäßlumina, Ersatz des freien Raumes durch Basalmembranabwärtz (B). K: Kapillare. BK: Bowman'sche Kapsel.

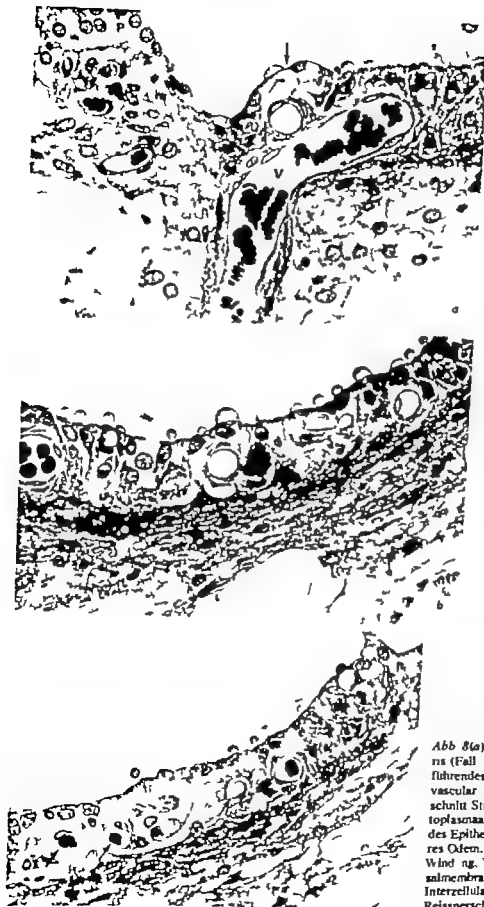


Abb. 8(a) Ausschnitt aus der Stria vascularis (Fall 1) P Prominentia spiralis \ abführendes venöses Gefäß (/) Ödem perivascular Basalwindung  $\times 580$  (b) Ausschnitt Stria vascularis Basalwindung, Protoplasmaabschnürungen an der Oberfläche des Epithels \ or allem basales perivaskuläres Ödem,  $\times 465$  (c) Stria vascularis zweite Windung, Verbreiterung der kapillären Basalmembranen (/) deutlich abgegrenztes Interzellularödem (+) nahe dem Ansatz der Reissner'schen Membran (RV)  $\times 415$



Abb. 9(a) Corti-Organ der zweiten Windung. Degenerative Veränderungen der Inneren Haarzelle (IHZ) bei normaler Struktur der Äußeren Haarzellen (AHZ). 485 (b) Corti-Organ der Basiswindung. Äußere Haarzellen intakt, innere Haarzelle pyknotisch (S) Synapse einer Äußeren Haarzelle haften an Tectoralmembran.  $\times 485$



Abb. 10 Ausschnitt aus dem Ganglion spirale der Basiswindung. Normale Struktur, fehlende Myelinisation ist nicht methodisch. 400



Abb 11 Elektronenoptischer Ansicht aus der Stria vascularis der Basalwindung. Deutlicher als im Lichtmikroskop wird das ausgedehnte Interzellularödem geschlossener epithelialer Oberfläche. Auch die Basalzellschicht ist aufgedockt (BZ)  $\times 4510$

weiteren Windungsanteilen voll erhalten in der Basalwindung fehlten die inneren Haarzellen in den höheren Windungsanteilen waren die inneren Haarzellen vacuolig aufgetrieben und im Zustand der Degeneration. Die Tunnelradialfasern waren voll erhalten. Die Spiralganglienzellen zeigten sowohl bezüglich der charakteristischen Morphologie als auch bezüglich der Gesamtpopulation keine auffallenden Veränderungen (Abb 9a b 10).

Elektronenmikroskopisch wurde im Bereich der Stria vascularis das interzelluläre Oedem deutlicher als im Lichtmikroskop auffallend war jedoch eine starke Verdickung der Basalmembranen der Striagefäße (Abb 11 12). Das letzte Tonschwellenaudiogramm drei Monate vor dem Tode zeigte eine gering bis mittelgradige fast pancochleäre Schwerhörigkeit die Diskrimination betrug 100% das Recruitment war positiv. Von seiten des Vestibular

organs wurden keine Beschwerden beklagt ein Spontan oder Provokationsnystagmus bestand nicht.

## DISKUSSION

Vergleicht man diese Befunde beim Alport Syndrom mit den Ergebnissen der experimentellen Masugi Nephritis (Quick 1975 Arnok et al 1976) so haben beide folgendes gemeinsam: Antikörper gegen Basalmembranen bei der experimentellen Masugi Nephritis bewirken eine Anschwellung der Basalmembranen der Nierenglomerula und ein Zusammenfließen der epithelialen Ausläufer der Nierenglomerula. Ferner kommt es im Innenohr aus schließlich zu einer immensen Verdickung der Basalmembranen im Bereich der Striakapillaren. Veränderungen am Cortischen Organ oder an den Ganglienzellen des Ganglion spirale wurden im Kurzzeitversuch nicht gesehen.



Abb. 12 Lamellierung und massive Verbreiterung der Basalmembran einer Stria-  
pillare perivaskuläres Ödem, an einer Stelle zieht die Basalmembran zwischen die  
Aussäule der Marginalzellen (P). 25 000

Beim Alport Syndrom kommt es ebenfalls zu Beginn der klinischen Manifestation zu einer Verbreiterung der glomerulären Basalmembranen mit Verschmelzung der epithelialen Flüsschen. Im Spätstadium des nephrotischen Syndroms sind die Basalmembranen extrem verdichtet und aufgetrieben; daneben findet man eine Proliferation der Epithelzellen. Im Bereich der Stria vascularis fällt ein interzelluläres Oedem nahe der Prominentia spiralis und nahe dem Ansatz der Reissner'schen Membran auf; daneben eine deutliche Verdichtung und Verdickung der Basalmembranen um die Stria-gefäße. Schließlich beobachtet man degenerative Veränderungen der Epithelzellen, die bei schwerem Verlauf der Erkrankung zu einer kompletten Degeneration der Stria vascularis führen. Diese Veränderungen erscheinen zunächst die stärkste Auswirkung auf die innere Haarzelle zu haben, da diese vorwiegend in den basalen Windungsanteilen deutliche morphologische Schäden aufweisen. Die Spiralganglienzellen dagegen waren in dem vorliegenden Fall intakt. Erst im Spätstadium der Erkrankung, wenn die komplette Stria-

generation eingetreten ist, wird auch ein allgemeiner Haarzellverlust und eine womöglich retrograde Degeneration der Spiralganglienzellen erkenntlich. Entsprechend findet man im Tonschwellenaudiogramm zu Anfang der Erkrankung einen Hochtonverlust; der Schallempfindungsverlust im Mittelfrequenzbereich mag bei 100%igem Diskriminationsvermögen als Folge der frühzeitigen strahlen Schädigung aufzufassen sein.

Da nach Spichtin & Mihatsch (1979) die Erkrankung meist in früher Kindheit mit Mikro- oder Makrohämaturie sehr oft im Anschluß an einen respiratorischen Infekt (33%) der oberen Luftwege beginnt und die Veränderungen am Innenohr offensichtlich eine Spätfolge der Erkrankung darstellen, ist zu überlegen, ob die Innenohrveränderungen beim Alport Syndrom nicht Folgen der primären Nierenschädigung sind und weniger als Ausdruck einer genetischen Determination aufzufassen sind. Ob dabei diese Schädigung wie beispielsweise bei der experimentellen Masugi-Nephritis, Folge einer durch exogene Beeinflussung induzierten immunologischen Reak-

tionsweise ist (gemeinsame Antigenizität von Nierenglomerulabasalmembranen und Basalmembranen der Striagefäße) oder aber die Nierenerkrankung am Beginn der Erkrankung steht und erst im Laufe der Zeit auf immunologischem oder direkt toxischem Wege (vergl. Oda et al. 1974) das Innenohr beeinflusst wird kann vorerst nicht entschieden werden. Es spricht manches dafür, daß beim Alport Syndrom immunologische Reaktionsmechanismen ablaufen. Es wäre auch vorstellbar, daß bei dieser Erkrankung ein recht ähnlicher Pathomechanismus vorhanden ist wie er derzeit für den Rheumatismus (vergl. Laine 1979) oder für bestimmte Formen des Diabetes erörtert wird. Gerade die Tatsache, daß Infekte die primäre Nierensymptomatik auslösen (Zollinger & Mihatsch 1978) deuten auf eine exogen induzierte Autoimmunkrankheit hin, die – da familiär gehäuft auftretend – einer gewissen Prädisposition bedarf (ähnlich Diabetes, Rheumatismus).

Überblickt man die anstehende Literatur über die wenigen sporadischen Beobachtungen bei Fällen von Alport Syndrom (Übersicht bei Schuknecht 1974), so vermißt man regelmäßig zu beobachtende pathologische Veränderungen am Innenohr, wogegen die Veränderungen an der Niere als charakteristisch eingeordnet werden können. Unter den Innenohrveränderungen findet man Ganglienzellverlust, Atrophie des Ligamentum spirale, Haarzellverlust, Striaatrophie oder basophile Ablagerungen in der substrialen Zone. Gerade letztere sind auch bei verschiedenen anderen Innenohrerkrankungen beobachtet worden (vergl. LaVonne, Bergstrom et al. 1979).

Es läßt sich abschließend feststellen, daß die schwersten Veränderungen am Innenohr bei nierenbedingtem Grundleiden regelmäßig an der Stria vascularis zu suchen sind. Man darf daraus folgern, daß bestimmte Nierenerkrankungen zuerst zu einer Schädigung der Stria vascularis führen, wobei der Pathomechanismus sowohl auf direkt toxischem wie auch auf immunologischem Wege ablaufen kann. Die weiteren Veränderungen am Innenohr wie

Untergang von Haarzellen oder Verlust cochleärer Neurone sind entweder sekundäre Folgen der Striadegeneration oder unmittelbare Folgen nierenbedingter Störungen des Innenohrmetabolismus. Darauf deuten vor allem die Beobachtungen von Schreiner oder McCabe (persönliche Mitteilungen) hin, die beim Alport Syndrom und anderen Nierenerkrankungen die mit Innenohrschwerhörigkeit einhergehenden unter immunsuppressiver Therapie (z. B. Cortison) einen Wiederanstieg der Hörfunktion beobachteten. Dies ist nur möglich, wenn zum Zeitpunkt des Therapiebeginns die sensorischen und neuronalen Komponenten des Innenohres noch intakt waren, da neuronale Elemente im Gegensatz zur Stria vascularis nicht mehr regenerationsfähig sind. Diese Beobachtungen sprechen auch gegen eine genetisch determinierte Innenohrdegeneration beim Alport Syndrom.

## RÉSUMÉ

Les lésions histologiques du rein et de l'oreille interne sont démontrées dans deux cas de syndrome d'Alport. Les altérations de l'oreille interne sont représentées au voisinage des capillaires striales et dans les cellules d'organ de Corti. Les lésions de la cochlée sont – après notre impression – la conséquence de la glomérulopathie.

## SUMMARY

The histological findings in two cases of Alport's syndrome are presented. Damage within the inner ear is mainly represented in the vascular stria region and it seems that the inner ear damage pattern tallies with the renal alterations. Glomerular basement thickening and splitting are the first and pathognomonic findings together with confluence of the epithelial feet. Later on, the increase in volume in the epithelial cells and the tremendous enlargement of the glomerular basement membranes leads to an obliteration of glomerular capillaries. In the first described case a subtotal loss of stria vascularis seems to be the reason for a degeneration of the inner and outer hair cells as well as of some spiral ganglion cells. The second case revealed only a slight edema of the stria vascularis and apical protrusions of cytoplasm sometimes vacuolated. Whereas in the basal turn most of the inner hair cells had disappeared in the upper turns we observed a slight degeneration of the inner hair cell but intact outer hair cell system. Although in Alport syndrome there are – according to the literature – no constant findings, our observations allow us to assume that inner ear damage in the cochleo-renal syndrome is secondary to the kidney disease.

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## DISCUSSION

- Krelemen to Arnold. Emphasizes scepticism regarding the dilatation theory in Meniere and other conditions. Temporal bone histology requires an equally careful preparation whether for light or electron microscopy.



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## PURETONE THRESHOLD ESTIMATION FROM ACOUSTIC REFLEX THRESHOLDS—A MYTH?

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**Abstract.** There have been repeated claims in the literature that hearing thresholds can be estimated by means of acoustic reflex threshold (ART) measurements. We have evaluated this thesis in depth by examining the statistical relationships between conventional puretone thresholds and ART for tonal and broadband stimuli in large sample (1707) of adult patients with sensorineural hearing loss; they were compensation claimants for noise-induced loss. All relevant data were stored in a computer database and analysed using the Statistical Package for the Social Sciences (SPSS). The relationships were assessed from two distinct standpoints, dependency of ART upon puretone thresholds and prediction of audiogram features using ART. The average tonal ART is constant up to 70 dB average loss, and thereafter increases rapidly. The broadband noise ART increases steadily for average losses of 10 to 85 dB. The convergence principle therefore valid up to 70 dB in this population. The predictor suggested by Niemeyer & Sesterhenn does not fit the data. The single ART best predicts average loss is that for line noise but multiple regression prediction using the line noise and 500 Hz ART is preferable. Prediction accuracy is dependent upon audiogram slope but the latter cannot be predicted usefully from the ART measures used. None of the predictors studied is adequate for medico-legal assessment and the method is probably not sufficiently accurate for clinical use in adults.

Prediction of auditory puretone thresholds (PTT) from acoustic reflex threshold (ART) measurements has intrigued auditory scientists since it became practical to measure the acoustic reflex. Potentially it provides an objective technique which is cheaper, quicker and simpler to perform than various methods of electric response audiometry (ERA).

Niemeyer & Sesterhenn (1974) first proposed an empirical prediction equation for the PTT which depends upon a systematic decrease in the difference between wideband and puretone ARTs with increasing hearing

loss. This method has been expanded by various authors (Jerger et al. 1974; Handler & Margolis 1977; Keith 1977; Van Wagoner & Goodwine 1977; Miller et al. 1976).

The present authors take issue with many of the published reports.

Firstly, there is relatively little use of statistical methods. Prediction, for example by multiple regression analysis or by discriminant analysis, is a powerful and well developed area of statistical theory and practice. These methods provide a meaningful way of quantifying predictor performance and force explicit mathematical modelling of the phenomena. Empirical approaches, on the other hand, are prone to subjective bias and irreproducibility.

Secondly, there seems to be a general lack of rigor concerning errors in prediction. If a prediction of a numerical loss value is made, then the method can be evaluated in terms of bias and variability of predictive errors.

Thirdly, we disagree with the concept of categorization of hearing loss data, used by several authors, on the grounds that it is simplistic. The intent of a prediction method is to obtain some estimate of hearing loss; the latter is of the highest possible measurement strength, namely a ratio scale variable. Average hearing loss, for example, is not naturally categorical and to impose a categorization is to degrade it, with loss of information. Statistical methods for deriving eval-

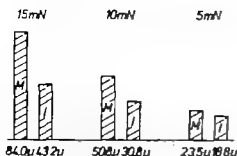


Fig. 3. Average value.

Static examinations into the stability of the malleus-incus joint were also highly conclusive. It is a well-established and generally recognized fact that this joint is not flexible and of no importance for the transmission of oscillations. It was possible to clearly check this stability and/or flexibility. If, for example, a force is applied at two points with the same lever arm (Fig. 1 upper left) the displacement must be the same—if the joint is inelastic. However, tests in 10 temporal bone preparations showed differences at forces of 5, 10 and 15 mN. The malleus could be moved more than the incus, and the differences were the more pronounced the greater the forces applied were (Fig. 3). From this we can see that this joint is by no means inelastic when such forces are applied. If we reduce the force to 2 mN, we still can measure a displacement difference of 1 to 1.2, only at 1 mN were the displacements of malleus and incus equal. From this we can conclude that if forces of below 1 mN act there is no transmission loss, which however can be certainly assumed to exist if the force is greater.

Static measurements cannot be compared with dynamic ones without reservation, but we can compare the forces which are active. Thus 1 mN corresponds to 100 dyn, which—related to the tympanum of about 50 mm<sup>2</sup>—corresponds to a volume of 170 dB. This figure tells us that within the human auditory area there is no loss of oscillation energy in the malleus-incus joint. However, in the case of great sound intensities, i.e. above the pain threshold, the joint becomes more and more elastic, whereby the oscillation of the incus does not increase in the same extent as that of the malleus. We may interpret this as being a function of sound protection.

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## DISCUSSION

*Tonndorf to Cancura:* There is a great need for static measurements for the construction of middle-ear prostheses.

There is no dispute between Békésy and us with respect to the mode of malleus displacement, only to that of the tympanic membrane.

I would urge you to repeat your measurements in living cats and then follow up after sacrifice. Impedance changes rapidly after death and so should the material constant.

There is movement in the incudomalleal joint at  $f > 100$  Hz, but it is amplitude-independent.

*Cancura (Reply):*

Influences caused by ageing or drying were naturally examined. No significant differences were ascertained as long as the preparations were kept under optimal conditions, and it was easily possible to reproduce all measurements. Variations occurred only when the ligaments started to disintegrate, e.g. through putrefaction.

Table 1 Summary statistics for puretone AC thresholds

| Frequency<br>(kHz) | Right ear |      |        |      | Left ear |      |        |      |
|--------------------|-----------|------|--------|------|----------|------|--------|------|
|                    | N         | Mean | Median | S.D. | N        | Mean | Median | S.D. |
| 0.5                | 1 203     | 25.2 | 22.7   | 13.4 | 1 203    | 25.8 | 23.5   | 13.4 |
| 1.0                | 1 206     | 36.3 | 36.3   | 15.2 | 1 704    | 36.9 | 37.7   | 15.8 |
| 2.0                | 1 204     | 52.1 | 53.6   | 14.3 | 1 203    | 54.1 | 55.4   | 13.9 |
| 3.0                | 1 203     | 64.9 | 63.4   | 13.8 | 1 198    | 64.2 | 64.4   | 13.4 |
| 4.0                | 1 195     | 68.3 | 68.1   | 14.4 | 1 195    | 69.2 | 68.6   | 13.6 |
| 5.0                | 1 184     | 67.4 | 68.2   | 20.2 | 1 182    | 68.5 | 69.4   | 19.1 |

is that the reflex plane is divided by two intersecting line segments: apparently the slope of the line segments and the point of intersection are determined by inspection, not by any formal optimisation method. This makes statistical interpretation difficult.

Johansen et al (1976) stated that ARTs for puretones in patients with cochlear deficits are largely independent of the audiometric threshold, and they queried the logic behind the Niemeyer-Sesterberna prediction formula. However they showed a clear positive association between average hearing loss and the ART for white noise, but they did not express this in terms of a linear regression equation. They showed a scattergram for hearing loss against the difference between puretones and white noise ARTs which showed very little association. It may therefore be inferred that inclusion of the puretone average ART in a prediction equation may not contribute to the prediction accuracy and may even degrade it.

Keith (1977) reviewed the work of Niemeyer & Sesterberna and Jerger et al, noting that audiogram slope prediction by reflex methods is equivocal and that prediction methods based on white noise reflexes were as accurate as those which also included puretone ARTs.

Hall (1978) compared two versions of the Jerger predictor with an unpublished regression predictor reported by Baker & Lilly (1976). The predictive accuracy of the three methods was similar and decreased systematically as a function of increasing age. The performance of the Jerger predictors was de-

graded in the presence of minor abnormalities of the tympanogram.

In a comprehensive report Jerger et al (1978a) examined the effect of age, degree of hearing loss and audiogram configuration upon the ARTs to puretone and wide-band stimuli. They found that all three variables influenced the reflex relations and that their effects were interactive. Jerger et al (1978b) reported the effects of age on reflex prediction. They found that predictive accuracy was substantially better in a group of children than for either young or old adult groups.

#### Present study

We attempted to model existing and new schemes in order to evaluate the efficacy of ARTs in hearing threshold determination of a specific population—adults with presumed noise-induced hearing loss (NIHL). From this group 1207 patients referred to our clinic (MSH) for assessment between January 1975 and January 1978 with reliable puretone audiograms and bilateral reflexes to wideband noise were selected as subjects. Data were analysed on the University of Toronto IBM 3033 computer primarily using the Statistical Package for the Social Sciences (SPSS).

**Reflex measurement methods.** The ART measurements considered here were obtained using standard clinical methods. Contralateral ARTs were obtained to tonal stimuli at frequencies of 250, 500, 1000, 2000, 3000 and 4000 Hz, and to unfiltered, low pass and high pass filtered white noise. The 3 dB point was 600 Hz for both filters. The units for the tonal

uating or comparing prediction formulae are far more comprehensive if the predicted quantity has ratio strength

The points made above and others may be applied to critical review of some of the major publications in this area

#### *Niemeyer & Sesterhenn (1974)*

The basic prediction principle was established by this brief but effective report. The authors stated that the prediction formula was

$$\text{ARTAV} - \text{PTAV} = 2.5(\text{ARTAV} - \text{ARTWN})$$

where ARTAV is the average ART to pure tones, PTAV is the average pure tone threshold and ARTWN is the ART to white noise in dB HTL.

The above form of the equation emphasizes the convergence of PTAV and ARTWN to ARTAV at different rates as PTAV increases. It can be rearranged to

$$\text{PTAV} = 2.5\text{ARTWN} - 1.5\text{ARTAV}$$

Niemeyer & Sesterhenn stated this prediction equation without describing any formal optimisation method to derive it. They also expressed their sample size in terms of ears rather than individuals. This is a common practice, the most obvious effect of which is to inflate sample size. If estimates of variation of any auditory measure are based upon the number of ears alone, then between-ear and between individual variation will be confounded.

Niemeyer & Sesterhenn noted that prediction was less accurate if the hearing loss was sloping, but they did not quantify this observation. Since it seems unlikely that the slope of the hearing loss profile would be known in advance, the relevance of a prediction equation restricted to flat losses is difficult to assess. They noted that sloping hearing losses are associated with an increase of the ART for high pass filtered noise relative to that for loss pass noise, but the relations were not quantified.

Jerger and his colleagues (1974) in a large

sample study gave considerable impetus to subsequent research on ART prediction methods. They introduced the concept of predicting four categories of hearing loss severity utilizing an empirical rule based on Niemeyer & Sesterhenn's work which appears to have strongly influenced subsequent thinking. They considered this justifiable because the prediction is affected by the slope of the audiometric contour, and because in a screening environment the accuracy associated with a numerical prediction is deemed unnecessary. Neither of these two reasons is sound. A method of predicting categories of hearing loss severity will be based upon decision rules utilizing the same underlying measures as numerical predictor. Thus categorization will also be affected by the slope of the audiometric contour, although it may not be so obvious due to the degradation imposed by the categorization. Predictive errors which are called moderate by Jerger et al can be as large as 84 dB HL! (Johnsen et al 1976).

The prediction rule developed by Jerger et al is based upon clinical experience, and the results are expressed as a matrix of observed and predicted categories. The observed counts or proportions in each of the matrix cells are only estimates of the true counts or proportions. No information is given concerning the variability of these estimates. So far as the present authors are aware, this is also true of all subsequent papers in which the data are categorized.

Handler & Margolis (1976) partitioned hearing loss severity into two categories: normal and abnormal. Prediction is based upon the position of the patient's reflex measures in two-dimensional plane: one axis of which is puretone ART, the other a broadband/puretone ART ratio. Both dimensions contribute to the separation between normal and abnormal groups. This method makes explicit use of puretone ART elevation, in addition to the bandwidth effect. Information is lost because the hearing loss scale is merely dichotomized. A further limitation of this empirical method

Table IV Pearson  $r$  values for various single ARTs Right ear

| ART type | 0.5  | 1    | 2    | 4    | WN   | LPN  | HPN  |
|----------|------|------|------|------|------|------|------|
| 0.5 kHz  | 1.00 | 0.68 | 0.40 | 0.25 | 0.66 | 0.65 | 0.36 |
| 1        |      | 1.00 | 0.61 | 0.32 | 0.74 | 0.71 | 0.45 |
|          |      |      | 1.00 | 0.49 | 0.61 | 0.55 | 0.54 |
| 4        |      |      |      | 1.00 | 0.41 | 0.37 | 0.51 |
| WN       |      |      |      |      | 1.00 | 0.85 | 0.65 |
| LPN      |      |      |      |      |      | 1.00 | 0.64 |
| HPN      |      |      |      |      |      |      | 1.00 |

high-pass noise ART shows poorer correlation at low frequencies but has the highest association with 4 kHz of the three noise types

(b) *Relationships between ARTs and behavioural thresholds* Consider any pair of measures one of which is an ART the other a behavioural threshold. These can be represented on a bivariate plane such that observed values of these pairs will be points in the plane. The pairs constitute a bivariate random variable which will be distributed over the plane.

Some correlation coefficients for behavioural and acoustic reflex thresholds to pure tones are shown in Table V. The ART to 2 kHz, for example, is correlated significantly with the PTT at that frequency. However the correlation is extremely weak even though the relationship is highly significant statistically. That is to say knowledge of either kind of threshold would give almost zero predictive power for the other kind of threshold. This was true of all other PTT-PT ART relationships studied.

The correlations of noise ARTs and the PTTs are shown in Table V. For the white noise stimulus the correlations are highly significant and fairly uniform over the frequency range. For lowpass noise and highpass noise the trends are as expected: the lowpass noise ART is more highly correlated with low stimulus frequencies and the highpass ART with high stimulus frequencies. For the puretone average, the three noise reflexes

correlate almost equally. These correlations are among the highest in the table.

(c) *Dependency versus prediction of PTT from ART* Since behavioural puretone thresholds are so fundamental it is natural to ask to what extent the various ARTs depend upon them. To answer this and simultaneously to examine in more detail the nature of the relationships which underlie the crude correlation coefficients the behavioural threshold must be treated as an independent variable and the ART as a dependent variable. It is then necessary to examine the properties of the conditional distributions of the ARTs given various values of the behavioural thresholds. The important point is that in studies of dependency it is the conditional properties of the ART which are examined.

In the process of prediction of behavioural thresholds from ART measurements an entirely different view of the bivariate distribution should be taken. In this case it is necessary to regard the ART as the independent variable and the behavioural threshold as the dependent variable. Predictive functions must be based upon the conditional properties of the behavioural threshold given values of the ART. This sharp distinction is not usually brought out in reports concerned with hearing loss prediction from ART measures.

These points are exemplified in Fig. 1 showing a cross-tabulation of the ART to white noise and the average PTT at 0.5-2

Table V Pearson correlations of ARTs and behavioural thresholds Right ear

| ART type | Behavioural threshold frequency |       |      |       |              |
|----------|---------------------------------|-------|------|-------|--------------|
|          | 0.5                             | 1.0   | 2.0  | 4.0   | Ave<br>1-2-4 |
| 0.5      | 0.07                            | -0.10 | 0.06 | -0.05 | 0.05         |
| 1.0      | 0.13                            | 0.07  | 0.05 | 0.01  | 0.09         |
| 2.0      | 0.10                            | 0.10  | 0.20 | 0.14  | 0.18         |
| 4.0      | 0.01                            | 0.02  | 0.08 | 0.22  | 0.11         |
| WN       | 0.21                            | 0.14  | 0.19 | 0.15  | 0.24         |
| LPN      | 0.20                            | 0.14  | 0.16 | 0.09  | 0.20         |
| HPN      | 0.11                            | 0.11  | 0.25 | 0.23  | 0.24         |

Table II *Percentage of cases with measurable ARTs*

| ART type | Right ear | Left ear |
|----------|-----------|----------|
| 0.5 kHz  | 99.0      | 99.3     |
| 1.0      | 98.7      | 98.8     |
| 2.0      | 91.9      | 90.4     |
| 4.0      | 60.5      | 56.8     |
| LPN      | 94.5      | 95.7     |
| HPN      | 86.9      | 84.9     |
| WN       | 100.0     | 100.0    |

ARTs are dB HL re ANSI 1969 and dB SPL for the noise ARTs

The reflex thresholds were measured on a Madsen Electro Acoustic Impedance Bridge Model Z0-72.1. Reflex responses were recorded on a Gould 2-channel strip recorder.

## RESULTS AND DISCUSSION

The selected sample comprised 1207 cases mean age 54.9 years range 27-78 years.

The puretone Air Conduction audiograms are summarized in Table I.

In Table II the proportions of measurable reflex thresholds are shown. It is clear that the proportion decreases monotonically with increasing frequency with a marked decrease at 4 kHz. The proportions for noise ARTs are also shown. Summary statistics for each reflex threshold are shown in Table III. If the ART is greater than the upper limit of the measuring

device it is ignored and the sample size changed accordingly. From Table III it is clear that the behaviour of the reflexes for the two ears is very similar. The mean ART increases monotonically with stimulus frequency. For the broadband stimuli the mean ART for LPN and WN are similar with that for HPN higher by about 7 dB. All tonal ARTs show similar values of variability which are slightly more than those for noise ARTs. It should be noted that the variability measured here is a combination of within subject and between-subject components presumably dominated by the latter.

(a) *Relationship between tone and noise ARTs*. A simple measure of the linear association between pairs of ARTs is the Pearson correlation coefficient. Significance tests are exact only if the underlying variates are bivariate normal which is not the case here. However inspection of the various  $r$  values shown in Table IV permits qualitative statements about relative strength of associations. All coefficients are highly significant statistically and all associations are positive. For the puretone ARTs the coefficients decrease as the frequency separation increases as is expected.

The white noise ART is strongly correlated with all puretone ARTs with a maximum at 1 kHz and a decline at 4 kHz. The low pass noise ART behaves similarly but with a greater decline as frequency increases. The

Table III *Summary statistics for ARTs*

| ART type | Right ear |       |        |      | Left ear |       |        |      |
|----------|-----------|-------|--------|------|----------|-------|--------|------|
|          | N         | Mean  | Median | S.D. | N        | Mean  | Median | S.D. |
| 0.5 kHz  | 1196      | 87.6  | 87.1   | 8.0  | 1199     | 87.4  | 86.6   | 7.9  |
| 1.0      | 1186      | 90.9  | 90.4   | 7.2  | 1194     | 90.8  | 90.4   | 7.3  |
| 2.0      | 1110      | 94.8  | 94.3   | 7.3  | 1097     | 95.1  | 94.6   | 7.5  |
| 4.0      | 731       | 99.3  | 99.5   | 7.5  | 666      | 99.6  | 100.2  | 7.8  |
| LPN      | 1142      | 93.4  | 93.3   | 6.7  | 1150     | 93.7  | 93.7   | 6.9  |
| HPN      | 1050      | 100.4 | 100.5  | 5.8  | 1025     | 100.9 | 101.1  | 6.1  |
| WN       | 1207      | 93.5  | 93.1   | 6.4  | 1207     | 93.8  | 93.5   | 6.6  |

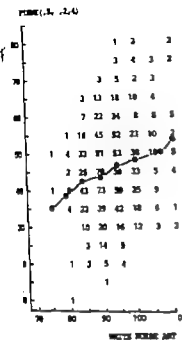


Fig. 3. Cross-tabulation of average ART at 0.5, 1, 2 and 4 kHz and the white noise ART. Circles are the conditional mean average PTT.

and thereafter shows a rapid rise. The white noise ART increases fairly linearly throughout the loss range. The convergence principle is therefore valid for losses up to 70 dB. These results are comparable with those obtained by Jerger *et al.* (1978a).

(c) *Prediction of audiogram features from ART measurements.* Most published reports on prediction of hearing level from ART measurements consider average hearing losses. Such an approach arises from an historical perspective or because of an underlying presumption that average hearing losses will somehow relate better to ARTs than will single puretone hearing loss measurements. The results in Table V show that this is not entirely true, but the noise ART/average loss correlations are among the highest in the table.

The predictability of the average puretone loss at 0.5, 1 and 4 kHz by single ARTs was examined. From Table V it can be seen that the white noise and high pass noise ARTs correlate equally well. The former was

Table VI. Conditional statistics analysis of variance and regression data for the average PT threshold at 0.5, 1, 2 and 4 kHz by ARTWN. PTAVE statistics.

| ART value | Mean | S.D. | N   |
|-----------|------|------|-----|
| 75        | 34.4 | 1.5  | 6   |
| 80        | 39.0 | 10.7 | 23  |
| 85        | 42.8 | 9.1  | 165 |
| 90        | 43.8 | 10.3 | 357 |
| 95        | 46.3 | 10.6 | 350 |
| 100       | 47.9 | 11.0 | 186 |
| 105       | 49.5 | 10.5 | 80  |
| 110       | 53.9 | 14.2 | 25  |

Analysis of variance:

Between groups:  $p$  less than 0.0001

Linearity:  $p$  less than 0.0001

Nonlinearity: NS

Simple linear regression:

$PTAVE = 7.6 + 0.4 \text{ ARTWN}$

Standard error of slope: 0.048.

Residual mean square: 109.7

F ratio 72.9  $p$  less than 0.0001

Number of cases: 1192.

selected for detailed study, because of its higher incidence (Table II).

The cross-tabulation of the average behavioural threshold at 0.5, 1, 2 and 4 kHz versus the white noise ART is replotted in Fig. 3 with the white noise ART as the abscissa. It can be seen from Fig. 3 that the conditional mean average loss increases linearly with increased ART (conditional statistics and regression data are given in Table VI). The key observation is that the range of the conditional means and therefore the approximate range of prediction, is only about 20 dB and the standard error of prediction is greater than 10 dB at any value of white noise ART. Therefore although the white noise ART does permit prediction of average hearing losses, the clinical utility of this prediction is extremely limited.

Clearly the conditional average loss distributions will depend upon the population under study. If this were restricted in range then the range of prediction would also be restricted. We do not consider this to be a serious objection to these data. The range of average losses



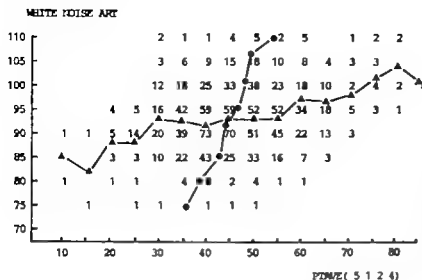


Fig. 1 Cross-tabulation of the white noise ART (dB SPL) and the average audiometric threshold at 0.5, 1 and 4 kHz. The latter is rounded to 5 dB steps. Triangles are conditional mean ARTs (column means); circles are conditional mean PTTs (row means).

and 4 kHz. These two variables form a bivariate plane quantised into 5 dB squares. The cell frequency is the number of times that co-ordinate pair was observed. If these were divided by the total number of observations then the height of each cell proportion above the bivariate plane would estimate the joint probability function. There is a tendency for large values of the white noise ART to be associated with large values of average hearing loss and vice versa. This is the reason for the highly significant positive  $r$  of 0.24 shown earlier in Table V. For dependency of the white noise ART upon the average hearing loss a natural statistic is the conditional mean ART for each column of the figure. These conditional means are indicated by triangles in the figure. It can be seen that there is a slight

but definite trend towards increasing ART as the average hearing loss increases.

For prediction of average hearing loss using the white noise ART the appropriate measures are the conditional means of the behavioural thresholds for each row of the figure that is for each value of the white noise ART. Such means are indicated as circles in the figure. It is extremely important not to confuse these two aspects of bivariate distributions. Not only are the conditional mean curves required in each case entirely different but it is perfectly possible that while good dependency may exist predictive relationships may be poor and vice versa.

In this report therefore dependency and prediction will be treated as entirely separate issues with major emphasis on prediction for the dependency of ARTs on PTTs has been commented upon by other authors and our findings are similar.

(d) *Dependency of ARTs on average hearing loss.* The basic rationale proposed by Niemeyer & Sesterhenn is convergence of broadband and average tonal ARTs as the hearing loss increases. This concept is valid for our data as shown in Fig. 2. This figure shows the conditional means for the average tonal ART at 0.5, 1 and 2 kHz and the white noise ART as a function of increasing average hearing loss at 0.5, 1, 2 and 4 kHz. The average ART is constant until a loss of 70 dB

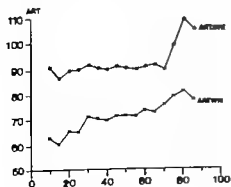


Fig. 2 Means values of ART against average PTT at 0.5, 1 and 4 kHz. Circles are the average ART at 0.5, 1 and 4 kHz. Crosses are the white noise ART.

## OBSERVED THRESHOLD

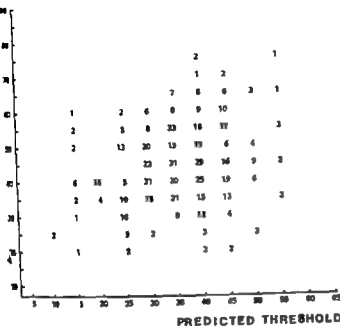


Fig. 5 Cross-tabulation of observed average PTT (ordinate) predicted average PTT (abscissa) from the Niemeyer-Sesterhenn predictor

### Comparative assessment of predictive formulae

The predictive performance of the Niemeyer-Sesterhenn formula was compared with prediction using white noise alone and with a multiple regression equation derived in this study. The Niemeyer-Sesterhenn formula incorporates the 4 kHz ART. Accordingly the formulae were compared in a sub-population of 719 patients having all appropriate reflexes.

In Fig. 4 the difference between the average ART at 0.5, 1, 2 and 4 kHz and the ART for white noise is cross-tabulated against the average ART minus the average puretone threshold at those frequencies. This corresponds to Fig. 3 in Niemeyer & Sesterhenn's original report (1974).

The solid line in Fig. 4 corresponds to their prediction equation which states that the abscissa is 2.5 times the ordinate. The data of this study do not follow that relationship: the bivariate distribution is clearly more horizontally oriented than that required by the formula. A substantial number of points are out

side the 20 dB limits especially for small values of the abscissa.

One way of visually assessing the performance of a prediction equation is to cross-tabulate the observed and predicted values. In Fig. 5 this cross-tabulation is shown for the Niemeyer-Sesterhenn predictor. If the predictions were perfectly accurate all the points would lie on a diagonal line with unit slope. The enormous scatter is clear from the figure. There is a tendency for predictive errors to be positive at low predicted values and negative at high predicted values. As well as a great deal of variation there appears to be systematic bias in prediction. These results differ in both location and scatter from those of Miller *et al.* (1976) but that study is both small-sample and contains a normal-hearing group.

Carrying out a simple linear regression of the four-frequency average hearing loss on the white noise ART in this sub-population gave a predictive formula shown in Table VII: the correlation coefficient was 0.22. The cross-tabulation of observed and predicted values is

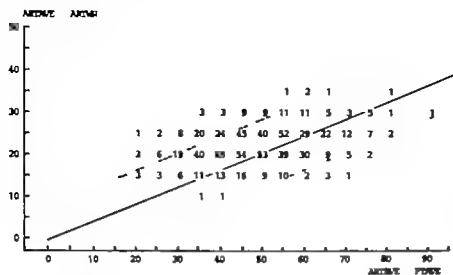


Fig. 4. Cross-tabulation of the components of the Nemjet-Sesterbenn predictor. ARTWAV is in dB HTL. The solid line represents perfect prediction. The dotted lines are 20 dB bounds on predictive error.

is from 10 to 85 dB and there is ample freedom within the data for a good predictive relationship to show itself. It does not, the main feature of the data being the enormous range of average losses observable for any given ART.

The results of this study were compared with those of Johnsen et al. (1976). Fig. 3 is analogous to the scatterplot of Johnsen et al. (in their Fig. 3). There are several remarkable differences between the two sets of results. The most obvious is that they obtained a correlation coefficient of 0.88, whereas that obtained in this study is 0.24. This is due to the rate of change of average hearing loss for a given change in ART being much greater in their study than in this one. The ranges of ART in the two studies are comparable and if, for example, the range from 75 to 110 dB is taken, then Johnsen et al. obtained a range of conditional mean from approximately 15 to 85 dB, that is a 70 dB range. In the present study the range is from approximately 35 to 55 dB, only a 20 dB shift.

Johnsen et al. have extended the range of ART measurements by incorporating the results from a normal hearing group. Such a procedure is inappropriate in prediction for two reasons. Firstly, as previously mentioned, prediction is a process of producing conditional statistics for hearing thresholds from ART measurements. If a normal-hearing

group is included, then clearly there has been some kind of selection process of the permissible value of the average hearing loss for that group. The conditional statistics are therefore biased. Secondly, an unevenly sampled range of abscissa values can lead to the classic hole in the middle error in regression analysis.

It is clear from the data of Johnsen et al. that even if the normal group is excluded, the correlation coefficient will be substantially higher than that obtained for this study. A possible reason is that the sample sizes at 105 and 110 dB in the Johnsen et al. study are extremely small compared to the present study. Any correlation or predictive function produced by the two studies will be more reliable for that with the larger sample size, that is, confidence intervals on estimates will be smaller. There is also the possibility that a large sample study will naturally reflect more variability sources than a small one, and that this will better reflect the variation in the population at large. Furthermore, the nature of the patient populations in the two studies may differ. In the present study, the aetiology is overwhelmingly NIHL, but Johnsen et al. did not specify the aetiology of hearing loss in their patients. Perhaps ART-PTT relationships are dependent upon subtle variations in pathology within the group of sensorineural hearing loss patients.

## OBSERVED THRESHOLD

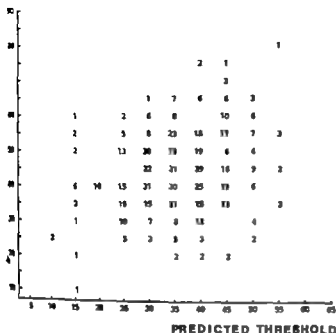


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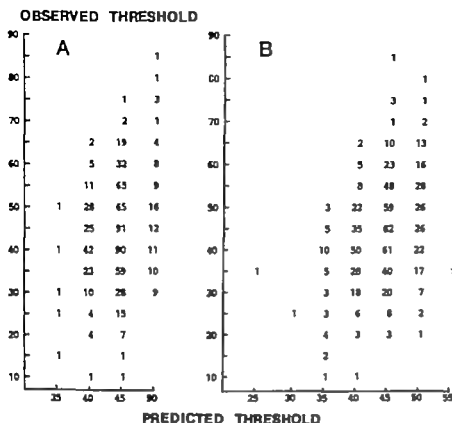


Fig 6 Cross-tabulation of observed (ordinate) and predicted (abscissa) average PTT for (A) linear regression on white noise ART and (B) multiple linear regression on white noise and 500 Hz ARTs.

shown in Fig 6a. Although there is no obvious bias, the scatter is comparable to that of the Niemeyer-Sesterhenn formula, and the predictive range is very restricted.

Applying multiple linear regression in this population and allowing the ARTs to white noise, 500 Hz, 1 kHz and 2 kHz as possible independent variables led to an equation involving only the white noise and the 500 Hz reflexes. This equation is shown in Table VII. This gave the best correlation coefficient of all the predictors discussed here.

The cross tabulation is shown in Fig 6b.

Table VII Comparison of predictive formulae in the subpopulation with measurable ARTs at 0.5, 1, 2 and 4 kHz.

|   |       |
|---|-------|
| Niemeyer-Sesterhenn<br>PTAVE=2.5 ARTWN-1.5 ARTAVE   | -0.24 |
| MSH data. White noise ART regression<br>PTAVE=0.43 ARTWN+5                                  | -0.00 |
| MSH data. Multiple regression on WN<br>0.5, 1, 2 kHz ARTs<br>PTAVE=0.31 ARTWN-0.45 ART500+9 | -0.34 |

There is no obvious bias, but the range is almost as severely restricted as for the white noise predictor. The standard error of prediction is still greater than 10 dB.

It should be noted that the Niemeyer-Sesterhenn formula is not a regression formula and therefore not subject to the constraint of producing the minimum mean squared predictive error. Such unconstrained formulae can be made to produce any desired range by simply adjusting the multiplicative constant. The occurrence of a good predicted range for any formula is only a positive attribute if other features such as bias or scatter are not compromised.

No matter what set of reflex measures is used to predict the puretone hearing loss, linear combinations of these measures do not contain substantial predictive power. Even the best of them, that is the multiple regression formula using the white noise and 500 Hz reflexes, with the correlation coefficient of 0.34, only suffices to explain less than 12% of the observed variation in average hearing loss. Although highly significant statistically, this is

Table VIII. Regression data for low and high slope groups

## White noise ART alone

|            |         |                    |           |            |
|------------|---------|--------------------|-----------|------------|
| All        | PTAVE = | 7.6 + 0.41 ARTWN   | $r = 0.4$ | $N = 1192$ |
| Low slope  | PTAVE = | -13.4 + 0.67 ARTWN | 0.33      | $N = 289$  |
| High slope | PTAVE = | 13.5 + 0.30 ARTWN  | -0.20     | $N = 389$  |

## Multiple regression on ARTWN, 500, 1K, 2K

|            |         |                               |            |
|------------|---------|-------------------------------|------------|
| All        | PTAVE = | 16 + 0.73 ARTWN - 0.45 ART500 | -0.33      |
| Low slope  | PTAVE = | -1.4 + 0.11 ARTWN - ART500    | -0.32      |
| High slope | PTAVE = | 29 - 0.42 ART1K + 0.40 ART2K  | $r = 0.32$ |

really very poor performance for a predictive function.

As Jerger's categorical analysis has been widely discussed the predictions of these various formulae were subjected to such an analysis using his categories. The errors of loss prediction by the Niemeyer-Sesterhenn formula, the white noise regression formula and the multiple regression formula were compared with those achieved by Jerger et al. (1974) and the results are shown in Table IX.

In view of the preceding discussion of the limitations of ART based predictors it seems that categorical analysis is misleading for the population under study.

## The effect of audiogram slope on prediction

In the original report by Niemeyer & Sesterhenn and in several subsequent reports it has been stated that the predictive accuracy of acoustic reflex measurements is less in high slope audiograms. This assertion has not been fully quantified in the published literature.

Two groups of patients were extracted from the overall population. Audiogram slope was

defined as the difference between the puretone thresholds at 4 kHz and 1 kHz. There were 289 low slope (10 to 25 dB) and 389 high slope (40-55 dB) patients.

The best single predictor variable in the whole population was the white noise ART (Table VI). The results of applying linear regression separately to the low slope and high slope groups are shown in Table VIII. The regression and correlation coefficients are quite different for the two groups. Conditional means and regression lines are shown in Fig. 7.

These data show that the accuracy of prediction of average hearing loss by the white noise ART is indeed affected adversely by audiogram slope. The difference between the regression coefficients for the two slope groups is disturbing since it means that the population is highly heterogeneous with respect to the predictor function that should be used. The predictor function for the whole population is clearly a compromise between that for the low slope and high slope groups and is not particularly appropriate for either group.

Table VIII shows the result of applying

Table IX. Comparison of errors for various prediction methods

| Error (%) | Niemeyer-Sesterhenn | White noise regression | Multiple regression | Jerger et al. (1974) |
|-----------|---------------------|------------------------|---------------------|----------------------|
| None      | 58                  | 6                      | 63                  | 60                   |
| Moderate  | 41                  | 38                     | 37                  | 36                   |
| Severe    | 1                   | 0                      | 0                   | 4                    |

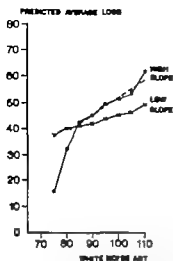


Fig 7 Conditional mean average PTT for low and high slope groups against the white noise ART. The dashed and dotted lines are regressions

multiple regression analysis to the low and high slope groups independently. It can be seen that the regression equations for the two groups are highly dissimilar to the extent that they do not even contain the same variables! It is interesting to note that the stepwise algorithm has not even selected the white noise ART for inclusion in the regression formula for the high slope group. It should be noted that the correlation coefficients are 0.37 for each group, which compares very well with the 0.33 for the overall population. This is an interesting result implying that it is possible to achieve a similar degree of predictive power in both groups, but that the predictor functions

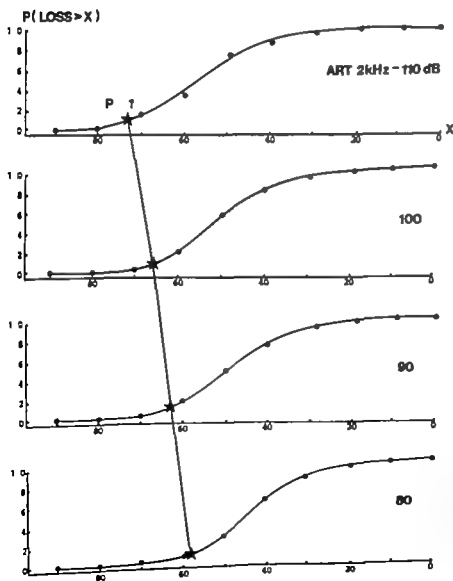


Fig 8 Estimated cumulative distribution functions for the PTT at 2 kHz ART takes the decade values indicated. The starts indicate the PTT which is exceeded in 10% of cases.

necessary to achieve this involve completely different sets of variables.

The decreasing effectiveness of the white noise reflex as a predictor of average hearing loss in the high slope group and the production of completely different multiple regression predictor equations for the low slope and high slope groups, make the use of ART prediction procedures even more problematic.

One possible solution to this is to develop prediction procedures in two stages: the first consisting of the prediction of slope and the second a prediction of severity. To this end the ability of reflex measures to predict audiogram slope was examined. The measure selected was the ART to high pass noise minus the ART to low pass noise. This was both correlated and cross-tabulated against the observed audiogram slope as measured by the difference between the 4 kHz and the 1 kHz puretone thresholds. The correlation coefficient obtained was only 0.22 and the cross-tabulation showed no appreciable diagonalization. It is concluded that this ART measure cannot serve as a useful basis in a multistage prediction process.

One final and simple examination undertaken attempted to relate values of PTT to ARTs for PTs. It has often been pointed out that if a recorded PTT were worse than a measured ART for that frequency the PTT must be in error (Alberti 1970). However the literature is not clear how much higher the ART must be than the PTT before the latter can be considered reliable. We investigated what level of PTT had a less than 10% chance of actually being exceeded for different decade levels of ART. The results for 2 kHz are shown in Fig. 8 where it can be seen that if the ART is 100 dB or greater the PTT has a 90% probability of being at least 35 dB better, whereas if the ART is 90 or 80 then the PTT may come to within 25 dB of the ART. If it comes closer the patient should be re-examined audiometrically before accepting the result.

The results of this study are disappoint-

ing. In adults objective predictive techniques are potentially of greatest use in medico-legal settings, particularly with large populations such as in screening industrial hearing loss claims. It is exactly such a population which has been tested and where all simple techniques appear to have no real quantitative value. ART measurement has a qualitative role to play both in site of lesion testing and in identification of hearing loss but no accurate quantitative predictions about hearing loss can be based upon it.

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## THE CRITICAL INTENSITY FOR OCCUPATIONAL NOISE

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**Abstract** Decades of medical examinations have shown that large-scale risks of hearing defects exist not only from 90 dB(A) upwards but even from 85 dB(A) upwards. This experience is confirmed by the analysis of 25 000 tone threshold audiograms and by the results of experimental hearing fatigue tests.

The critical intensity is of interest not only audologically but also from the viewpoint of occupational medicine. As is generally known, the noise to which people are exposed at their working places leads at first to a high tone hearing defect (Fig. 1) and later to a reduction in language hearing. Thus a certain proportion of persons exposed are affected by noise induced hearing defects.

Such hearing defects develop when the work noise exceeds the so-called critical intensity. If the noise level is lower, the hearing remains intact despite several hours of daily work. This fact explains the intensive endeavors aimed at finding a generally applicable value for this critical intensity.

For two reasons this has been difficult: 1) As opposed to other work-related organic defects, the human ear shows highly variable sensitivity to noise influences depending on the respective person; 2) so far, no hearing measurement results have been gained in large-scale examination series, nor have regular surveys of technical noise data been carried out at the work places. But it is only in this way that the hearing defects and hearing levels can be correlated.

The intensities referred to in medical literature are thus not based on the evidence of actual hearing losses but rather on the results of experimental hearing fatigue tests. Accordingly, the data vary between 70 and 105 dB. Only recently have we realized that the

actual value must lie between 80 and 90 dB(A). Although this margin amounts to only 10 dB, it gives rise to two important considerations.

### 1) An economic consideration

In a large number of enterprises, the noise level lies between 85 and 90 dB(A). If the critical intensity is fixed at 90 dB(A), this means a marked reduction of the costly supervision of hearing.

### 2) A medical consideration

The economic requirement must be met only if there is medical proof for the fact that noise levels cause work-induced hearing defects only upwards of 90 dB(A).

Series of audiometrical examinations in workers exposed to noise have been carried out in Austria since about 1957. Very early I made the observation that noise levels of below 85 dB(A) cause practically no hearing defects, while noise levels of 85 dB(A) cause the same defects as those of 90 dB(A). For this reason I recommended many years ago that the lower limit of the critical intensity be fixed not at 90 but at 85 dB(A). In Austria, the audiological examination program is based on this value as its guideline.

Recently, two projects were carried out at the audiology department of the First Otolaryngological University Hospital, Vienna. They confirm the pathogenicity of the 85 dB(A) noise level.

Sound-audiometrical measurement results obtained in about 25 000 workers exposed to different noise levels were subjected to analysis (Fig. 2). There are five groups of noise levels: below 85 dB(A), 85-88 dB(A), 88-91 dB(A), 91-97 dB(A), and over 97 dB(A).

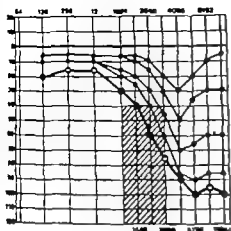


Fig 1

The study covers hearing losses at the most severely affected frequency of 4 kHz and such at the frequencies of 3 and 2 kHz. The calculations are made not on the basis of the arithmetic mean value but in percentiles. The breakdown of the data permits a listing of the hearing loss in the individual frequency ranges suffered or exceeded by a certain percentage of test persons. A percentile of 50 indicates that 50% of the random samples are below and 50% over this point. A percentile of 90 means that 90% of the hearing losses covered are below this value and only 10% are above. It

is above all the hearing losses suffered at 4 kHz which are of interest. As can be seen from the 50-percentile curve there is a slight increase at the 85 dB(A) level levelling off significantly toward 91 dB and even 97 dB. The hearing loss curve rises again more markedly only from a noise level of 97 dB upwards. This tendency can be seen even better in the 90-percentile curves. In line with the typical noise induced hearing loss the situation is similar at a frequency of 2 kHz, there are few changes however in the hearing losses suffered at 2 kHz. According to the results of this study the hearing defect break is to be found not at 90 but at 85 dB(A). The practical implications of this fact are seen in our own data material. Out of about 25 000 persons covered 23 446 are exposed to a noise level of 85 dB(A) and more (Fig. 3). Of them 59.6% work at noise levels lying between 85 and 90.6 dB(A). If in their case 90 dB(A) had been fixed as the decisive value for audiometric supervision a majority of them would not have been medically covered at all despite the same hearing risks.

Our second consideration relates to experimental studies. Today it is generally recognized that repeated noise induced hearing fatigue is responsible for permanent hearing

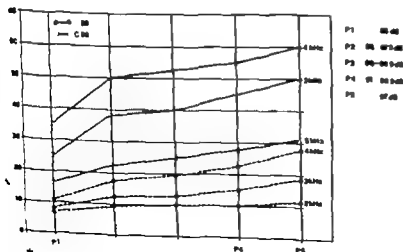


Fig 2 Hearing losses and exposure intensity

## THE CRITICAL INTENSITY FOR OCCUPATIONAL NOISE

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**Abstract** Decades of medical examinations have shown that large-scale risks of hearing defects exist not only from 90 dB(A) upwards but even from 85 dB(A) upwards. This experience is confirmed by the analysis of 25 000 tone threshold audiograms and by the results of experimental hearing fatigue tests.

The critical intensity is of interest not only audiologically but also from the viewpoint of occupational medicine. As is generally known the noise to which people are exposed at their working places leads at first to a high-tone hearing defect (Fig. 1) and later to a reduction in language hearing. Thus a certain proportion of persons exposed are affected by noise induced hearing defects.

Such hearing defects develop when the "work noise" exceeds the so-called critical intensity. If the noise level is lower the hearing remains intact despite several hours of daily work. This fact explains the intensive endeavors aimed at finding a generally applicable value for this critical intensity.

For two reasons this has been difficult: 1) As opposed to other work related organic defects the human ear shows highly variable sensitivity to noise influences depending on the respective person. 2) so far no hearing measurement results have been gained in large-scale examination series nor have regular surveys of technical noise data been carried out at the work places. But it is only in this way that the hearing defects and hearing levels can be correlated.

The intensities referred to in medical literature are thus not based on the evidence of actual hearing losses but rather on the results of experimental hearing fatigue tests. Accordingly the data vary between 70 and 105 dB. Only recently have we realized that the

actual value must lie between 80 and 90 dB(A). Although this margin amounts to only 10 dB it gives rise to two important considerations.

### 1) An economic consideration

In a large number of enterprises the noise level lies between 85 and 90 dB(A). If the critical intensity is fixed at 90 dB(A) this means a marked reduction of the costly supervision of hearing.

### 2) A medical consideration

The economic requirement must be met only if there is medical proof for the fact that noise levels cause work-induced hearing defects only upwards of 90 dB(A).

Series of audiometrical examinations in workers exposed to noise have been carried out in Austria since about 1957. Very early I made the observation that noise levels of below 85 dB(A) cause practically no hearing defects while noise levels of 85 dB(A) cause the same defects as those of 90 dB(A). For this reason I recommended many years ago that the lower limit of the critical intensity be fixed not at 90 but at 85 dB(A). In Austria the audiological examination program is based on this value as its guideline.

Recently two projects were carried out at the audiology department of the First Otolaryngological University Hospital Vienna. They confirm the pathogenicity of the 85 dB(A) noise level.

Sound audiometrical measurement results obtained in about 25 000 workers exposed to different noise levels were subjected to analysis (Fig. 2). There are five groups of noise levels: below 85 dB(A), 85-88 dB(A), 88-91 dB(A), 91-97 dB(A) and over 97 dB(A).

2) The analysis of 25 000 tone threshold audiograms made after hearing losses and noise exposure confirms the data gained in practical work.

3) Experimental studies show—as a consequence of the 85 dB(A) noise level—a lack of hearing recovery which is the ultimate cause of the permanent hearing defect.

## ZUSAMMENFASSUNG

Wie die jährliche kausale Begutachtung zeigt, bestehen stärkere Hörschädigungsrunden nicht erst ab 90, sondern schon ab einem Lärmpegel von 85 dB(A). Diese Erfahrung wird durch eine Analyse von 25 000 Tonsechshörumschreibungen und durch das Ergebnis experimenteller Gehörabdomungstest bestätigt.

F. Schm. etc.  
I. H. O. U. M. K. H. A. W. H. A.  
A. H. H. O. L. A. W. H. A. W. H. A.  
Austria

## DISCUSSION

*Speelman to Schm. etc.* Your first slide showed that there very little barely significant increase of damage risk between noise levels of 85 and 90 dB(A) whereas much greater increases exist between 75 and 80 dB(A) or 90 and 95 dB(A). What therefore is the reason I propose 85 dB(A) as permitted noise level first rather than 90 dB(A) as it is in Germany and Switzerland? The barely significant increase in damage risk will probably not

justify the enormous costs of reducing industrial noise emission by 5 dB.

*Townsend to Schm. etc.* W. In the US would also be happier with 85 dB(A) maximal level. However as you are fully aware this is not a scientific but a political decision. At the international level at the ISO (we'll have meeting in Delft) Sept. 26) I am happy to report (1) that the new standard will no longer give fixed values like 90 dB(A), but only guidelines so as to force the administrative authorities *agreed into action*, (2) the three speed frequencies will be increased to four 3 1.0 2.0, 3.0 HZ which will make the index more sensitive.

*Schm. etc. (Reply).*

The contributions made to the discussion, for which I am most grateful can be gathered together.

Of course the problem of fixing the critical intensity value at certain point is also a political question in that it touches upon the financial aspect. But as it involves the question of maintaining the health of the working population, it must be seen first and foremost as medical one. I too encountered difficulties with the competent authorities when many years ago I wanted to introduce 85 dB(A) as the critical intensity value to be generally observed in Austria. What I demanded at that time was based only on individual observations! The results which are now submitted should make it easier for the medical profession to convince the politicians through factual material.

As concerns the effect of noise-induced damage on sound-hearing, let me say the following: For the maintenance of speech hearing neither the auditory threshold for 3 kHz nor that for 2 kHz is of any decisive significance. As I was able to show only recently on the basis of a larger number of patients examined, the speech discrimination capacity depend above all on the auditory threshold loss at 1.5 kHz in the case of typical noise-induced damage.

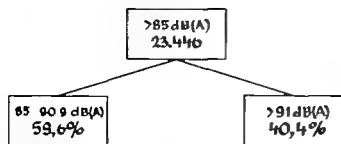


Fig. 3

defects. Therefore a study was carried out to establish the extent to which different noise levels can influence the recovery of hearing after a person's exposure to noise.

Two groups of young persons with normal hearing were exposed to different noise levels. At 3 kHz the first group showed a tone threshold shift (TTS<sub>2</sub>) of 32.5 dB (Fig. 4) the other of 17.1 dB. During the phase in which the recovery of hearing commences the ear is exposed again to noise of different secondary levels. As concerns our problem the noise levels of 75 and 85 dB are of main significance. Their influence on the tone threshold curve is observed over a period of 2 hours. When the TTS<sub>2</sub> was higher (32 dB) the hearing recovered rapidly in the first hour during the 75 dB noise; only in the second hour does it show some delay. The 85 dB noise however effects from the very beginning a smaller

degree of recovery which finally comes to a stop and in the second hour is followed by yet another hearing fatigue.

If the initial value of TTS<sub>2</sub> is not so high (about 17 dB) the different effect of the two secondary exposures to noise is even more pronounced. Even if much delayed signs of hearing recovery during the 75 dB noise the 85 dB level however prevents any recovery effect and even further intensifies the hearing fatigue.

Even if the effects of 85 dB noise on the unexposed organ of hearing are left out of consideration these results nevertheless suggest the following: An ear fatigued by noise exposure not only gives up the chance of recovering its hearing faculty but finally suffers from an even more severe hearing fatigue. This seems to explain why an increasing number of permanent hearing defects are found upwards of this noise level.

By way of summary we may state the following:

1) For many years medical examinations have shown that even noise levels of 85 dB(A) upwards cause marked hearing defects, partly even severe ones. In addition differences in the hearing loss cannot be found to exist in such persons when compared with persons exposed to somewhat higher noise levels.

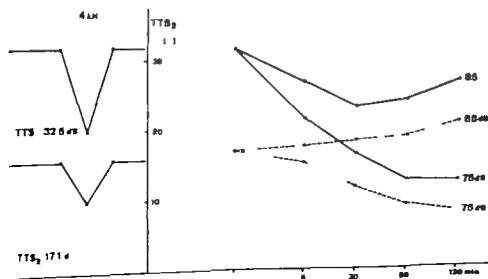


Fig. 4

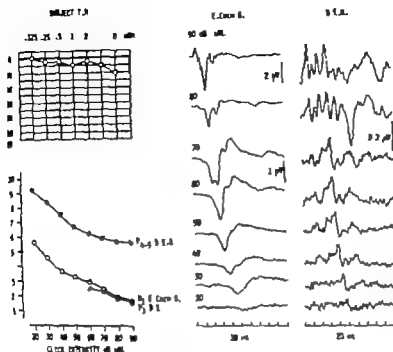


Fig 1 Typical results obtained with ECochG and BER in one normal subject. In these and all subsequent traces negativity at the active electrode is plotted downwards.

a conjunction (Coats & Martin 1977 Salomon & Elberling 1979) the combined use of ECochG and BER is not a common procedure at the clinic

This paper reports the first results we obtained with the combined use of ECochG and BER in the differential diagnosis of cochlear/retrocochlear disorders. Certain limitations of both methods are shown and the importance of their conjunction is emphasized

## METHODS

Forty-nine subjects were tested in this study. Nine of them were normal-hearing and constitute the normal group. Among the other 40 presenting with various degrees of hearing impairment a central tumour (generally acoustic neuroma) was confirmed by X-ray and surgical operation in 14 cases which constitute the retrocochlear group; the remaining 6 patients, mainly Menieres, showed no sign of retrocochlear disorder and were ranged in the cochlear group.

For TT ECochG the classical electrode positions were used: the active electrode rested on the promontory, a reference disc electrode was placed on the earlobe and the patient was grounded with a disc electrode on the forehead. For BER recordings small needles were inserted under the skin at the vertex (active electrode) and in the ipsilateral mastoid region (reference); the ground electrode remained as before on the forehead.

A broad-band click was used for acoustic stimulation. It was alternated in polarity and presented at a rhythm of 10 per second for ECochG and 70 per second for BER. It was delivered either in free field through a loudspeaker (Altec 808-8A) for ECochG or in close field through an earphone (TDH 49) for BER. Moreover for brainstem recordings a wide-band masking noise was sent to the contralateral ear through an earphone at about 30 dB below the click level in the test ear in order to avoid crossing over of click stimulation. In the majority of the patients in this study ECochG and BER were performed successively and click-evoked responses were recorded.

# TRANSTYMPANIC AND SURFACE RECORDINGS IN THE DIAGNOSIS OF RETROCOCHLEAR DISORDERS

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**Abstract** A series of patients presenting with subsequently surgically confirmed central tumour involving the auditory pathways were investigated using both transtympanic electrocochleography (TT ECoChG) and surface recordings of brainstem evoked responses (BER). While ECoChG allows a detailed study of peripheral function, BER allow the investigation of neural conduction up to the level of the inferior colliculus. Valuable information can be obtained from: (1) comparison of the amplitudes of the sensory and neural components of the ECoChG; (2) comparison of ECoChG, BER and auditory thresholds; (3) time interval measurements between the auditory nerve response (N) on the ECoChG and the inferior colliculus (wave P<sub>4-5</sub> on BER); (4) contralateral comparisons. Similar measurements performed in pure cochlear pathologies, mainly in Meniere's disease, yielded very significant differences.

The development of transtympanic electrocochleography (TT ECoChG) in the clinic during recent years has demonstrated that it can give much precise information on the functioning of the end organ (Aran 1973, Eggermont 1976). However, when information is needed on the condition of the auditory system beyond the cochlea, TT ECoChG is of limited value. Some characteristics of ECoChG responses have been related to the existence of retrocochlear disorders by several authors (Portmann & Aran 1972, Brackmann & Selters 1976, Odenthal & Eggermont 1976, Gibson & Beagley 1976); they are mainly a distorted pattern of the response: a shifted latency/amplitude function and a threshold discrepancy with subjective hearing. However, none of the proposed criteria allows a systematic detection of retrocochlear impairment, only a small proportion of these pathologies being thus diagnosed.

More recently, sound-evoked responses from the brainstem (BER) were shown to be recordable from surface electrodes and, although the responses are of low amplitude, some of their characteristics can be reliably measured even at low intensities. The study of retrocochlear disorders with BER has received much attention during recent years. As a first step, several measures of amplitude and/or latency of the various waves of BER have been proposed (Sohmer et al 1974, Thornton 1975, Starr & Achor 1975) and related to a variety of pathologies. However, among the later studies, the latency of the most prominent waves, the fourth and fifth peaks (P<sub>4-5</sub>), seems the most reliable and significant measure (Selters & Brackmann 1977, Stockard & Rossiter 1977, Clemis & Mitchell 1977, Coats & Martin 1977, Terkildsen et al 1977, Rosenhamer 1977). On the basis of BER recording alone, a high proportion of retrocochlear disorders was reported to be detectable as producing a significant delay in the latency of the P<sub>4-5</sub> wave. However, the values of P<sub>4-5</sub> latency are closely dependent upon the condition of the peripheral organ and latency shifts attributable to cochlear pathologies are often indiscernible and can lead to misinterpretations (Clemis & Mitchell 1977, Coats & Martin 1977, Brackmann & Selters 1979, Salomon et al 1979). To avoid such difficulties, combining of TT ECoChG with BER recordings is appropriate, since it gives precisely the latency of the neural activity at the cochlear level. Although both techniques give complementary information and appear more efficient

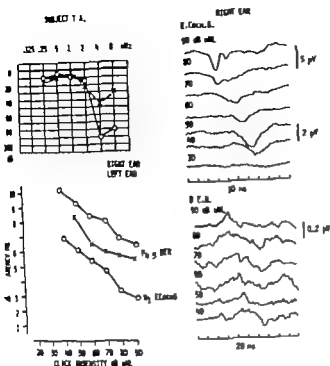


Fig 3 Typical example of the effects of various high-frequency hearing loss on BER responses. Compare the results with those in Fig 2.

measures to latencies. In Fig. 1 are presented the results from one normal subject. In all normal cases the latency/intensity curves of ECoG peak (N) and BER wave  $P_{1-2}$  were parallel and showed a latency difference of

about 4 ms (see also Fig. 5). At high intensity levels the latency of the first peak (P) in BER could be measured and was found to correspond to that of ECoG N, but for middle and low intensities it was impossible to detect.

#### Cochlear/retrocochlear cases

On the basis of ECoG results alone we found that the strongest indicator of a retrocochlear disorder was a threshold level better than could be expected from the subjective hearing revealed by the audiogram. Statistical studies relating the click threshold at ECoG to the subjective audiogram showed that it is a good indicator of subjective audiometric thresholds for middle frequencies (Eggermont, 1976). In all the subjects in this study we compared the ECoG click threshold with the best audiometric threshold for frequencies of 1, 2 and 4 kHz. The ECoG click threshold was better in 4 out of the 14 patients with a confirmed tumour; such a phenomenon was never observed in the group with cochlear impairment or in the normals. The pattern of ECoG response in itself did not appear sig-



Fig 4 Results from Mennere patient presenting with recruitment. Although the threshold is elevated the first peak (P) in BER recordings appears clearly.



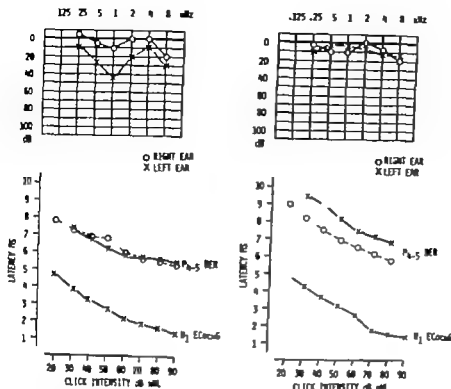
SUBJECT G.C.  
PENTENESUBJECT S.H.  
ACOUSTIC NEUROMA ON LEFT SIDE

Fig. 2 Comparison of ECoG and BER results in patients presenting with normal hearing or a moderate loss. In the case of unilateral neuroma, note the increase in latency of the BER response from the affected side.

tained from high intensities down to threshold in 10 dB steps.

A few cases were investigated with more sophisticated equipment allowing simultaneous ECoG and BER recordings using a multichannel system. In these simultaneous recordings we found it very interesting to use our stimulating paradigm earlier developed for the study of adaptation in ECoG. Trains of five clicks with an interclick interval of 10 ms thus stimulating at rhythm of 100 per second are presented every 100 ms i.e. at a rhythm of 10 per second. For ECoG this technique apart from the study of neural adaptation offers the possibility to precisely evaluate the sensory component (summing potential) of broad and abnormal responses. For BER adaptation can also be studied and in the same time five BER traces are obtained which can be compared in order to improve the detection of a response if needed. In these simultaneous ECoG and BER recordings clicks were delivered in free field by a loudspeaker and contralateral masking was applied through an ear phone.

In all conditions successive or simultaneous recordings the patients were slightly sedated with a Nembutal suppository at a dose of about 2 mg/kg in order to improve the quality of BER recordings.

## RESULTS

### Normal subjects

In the group of normals both ECoG and BER responses could be recorded down to very low intensities close to the normal hearing threshold for the click (see Figs 1 and 8). For ECoG responses were always very clear and both the amplitudes and the latencies could be reliably measured at all intensity levels and the waveform was easily recognizable. In BER recordings the series of waves appeared clearly at the highest intensities 70 to 90 dB above threshold but at middle or low intensities only one peak remained detectable that corresponding to the fourth and fifth waves (P<sub>4-5</sub>). We found that due to the poor signal to noise ratio amplitude measurements were not reliable and therefore we limited our

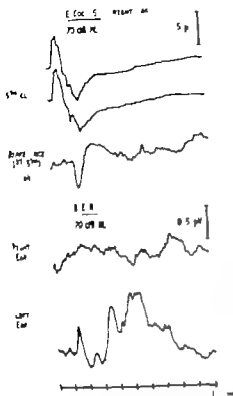


Fig. 7 ECoChG and BER responses in patient with neurectomy on the right side. Note that the ECoChG only clear response of abnormal pattern from the right chiasm and the evaluation of the neural and sensory components with the study of adaptation. In BER traces no response at all could be recorded from the right side whereas normal responses were clearly seen from the affected side.

If the patients presented recruitment and at high intensities P could be observed in 13 out of the 76 cases such an example is shown in Fig. 4. Only for those 13 subjects could the latency interval between the cochlear nerve response (P) and the late brainstem potential (P) be measured with BER alone.

Therefore in the majority of pathological cases ECoChG was necessary to evaluate the results of BER, as it permitted one to calculate the latency interval between the peripheral and brainstem responses. This time interval between ECoChG N and BER P<sub>4-6</sub> was found to be a very good criterion to differentiate between cochlear and retrocochlear disorders.

BER-ECoChG Click Threshold Difference

RETROCOCHLEAR  
COCHLEAR  
NORMAL

| BER-ECoChG    | 0-10 | 10-20 | 20-30 | 30-40 | 40-50 | 50-60 | 60-70 | 70-80 | 80-90 | 90-100 |
|---------------|------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| Normal        | 1    | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1      |
| Cochlear      | 1    | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1      |
| Retrocochlear | 1    | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1      |

Fig. 8 BER-ECoChG click threshold differences as observed in all the subjects in this study. Note that the results in normal and cochlear groups are concentrated in most cases around 0-20 dB. In contrast, noticeable proportion of retrocochlear cases presented significant threshold difference.

as shown in Fig. 5. All data points obtained for all subjects are presented in this figure and it can be seen that the latency interval is constant at about 4 ms for the normal and cochlear groups, whereas in cases of retrocochlear disorders it is increased. We found that a value of 4.5 ms was the best limit to distinguish cochlear from retrocochlear disorders. In 3 patients with a tumour we observed at some intensities a latency interval below the 4.5 ms limit. In such cases we considered the results over several intensities and found that for each patient the majority of values exceeded the 4.5 ms limit. These examples are shown in Fig. 6a. Using this criterion 2 cases, one cochlear and one retrocochlear, were misclassified. Their results are presented in Fig. 6b. The case of confirmed tumour presented an ECoChG click threshold better by about 25 dB than could be expected from the audiogram, which is a strong indicator of a retrocochlear impairment. The case of cochlear pathology was identified as a Meniere and presented no other sign of retrocochlear impairment.

In 4 patients of the retrocochlear group we could clearly record an ECoChG response on the affected side, whereas no response at all could be observed on BER recordings. In these subjects we could verify that normal BER waves were recordable when stimulating the unaffected side. Such an example is pre-

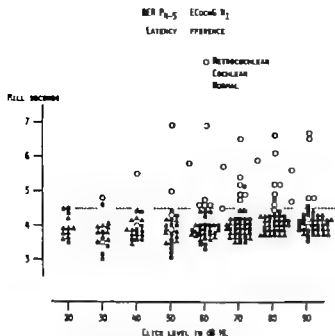


Fig 5 Latency interval between BER  $P_{4-5}$  and ECoG  $N_1$ . All data from all subjects are reported. Retrocochlear disorders clearly induce an increased latency interval. The dotted line represents the 4.5 ms limit separating at best retrocochlear cases from either cochlear pathologies or normals.

nificant to differentiate between cochlear and retrocochlear pathologies. In cases of retrocochlear pathology we observed mainly normal waveforms but also broad and abnormal patterns and in the group of cochlear disorders these types were also observed together with recruiting and dissociated patterns.

In pathological cases when the audiogram is normal or shows only a moderate loss the latency of  $P_{4-5}$  alone can serve to diagnose a retrocochlear disorder. Typical results obtained in two cases—one Meniere and one unilateral acoustic neuroma—are presented on Fig 2. It appears clearly that in these particular cases on the basis of BER alone a retrocochlear lesion can be detected as it produces an abnormal increase in the absolute latency of  $P_{4-5}$  as well as a clear interaural latency difference. Of course the latency interval between ECoG  $N_1$  and BER  $P_{4-5}$  is also abnormally increased.

In cases where the audiogram is noticeably altered—as was the case for most of the pa-

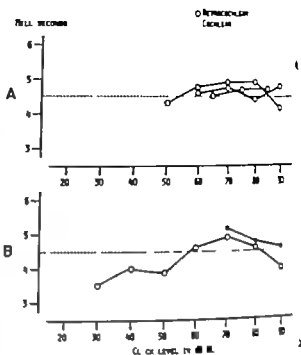


Fig 6 BER  $P_{4-5}$ —ECoG  $N_1$  latency intervals for certain particular subjects. (A) Subjects from the retrocochlear group who showed some values of latency interval below the 4.5 ms limit at some intensities. In each case, however, most values exceed the 4.5 ms limit. (B) Data points obtained in the 2 cases misclassified by the measure of latency interval.

tients in the cochlear and retrocochlear groups the latency of BER  $P_{4-5}$  is significantly modified which makes its interpretation difficult when considered alone. Such an example is presented in Fig. 3. This patient did not show any sign of retrocochlear pathology but due to the high frequency loss the BER latencies present the same abnormalities as those reported above in the case of acoustic neuroma. If the results of ECoG are taken into account no misinterpretation is possible since the  $N_1$  latency also appears delayed and the time interval measured between ECoG  $N_1$  and BER  $P_{4-5}$  can be identified as normal. As can be seen on the traces the first peak of BER ( $P_1$ ) is undetectable at any intensity and therefore the ECoG was essential for the interpretation of BER responses. In cases where the audiogram presented a hearing loss of 30–40 dB or more the  $P_1$  of BER could not be detected in the patients with a retrocochlear pathology whereas in the cochlear group most

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sented in Fig. 7 No BER  $P_1$  was detectable in recordings from the affected side so that with BER alone it might have been assumed that the peripheral organ was not functioning. In these cases ECochG was also essential to diagnose the retrocochlear impairment.

Finally, comparison of the click thresholds for ECochG and BER might be another index for the diagnosis of retrocochlear pathology. The threshold differences observed in all subjects in this study are reported in Fig. 8. For the normal and cochlear groups, threshold differences are clustered around 10–20 dB, whereas in the retrocochlear group 6 among the 14 subjects presented a threshold difference between 20 and 50 dB. Such threshold differences seem to be a valuable indicator of retrocochlear disorder but more data are needed to statistically validate its significance.

## CONCLUSION

The results presented in this study, although limited to a rather small number of subjects, clearly demonstrate the efficiency of combining both ECochG and BER recordings to diagnose retrocochlear disorders. Both methods alone have serious limitations but together they offer a variety of criteria which give them a very powerful approach. Some of these criteria are well established: an absence of BER waves with a clear ECochG response, an increased ECochG  $N_1$ –BER  $P_{1-4}$  latency interval and an ECochG threshold better than hearing threshold. Several other possibilities, particularly BER–ECochG threshold differences and interaural comparisons, need further investigation.

In cases of suspicion of retrocochlear disorder, the information afforded by these electrophysiological methods is now considered as important as the results of other classical methods.

## ACKNOWLEDGMENT

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## RÉSUMÉ

### Enregistrements transtympaniques et de surface dans le diagnostic des lésions rétrocochléaires

Tandis que l'électrocochléographie transtympanique (TT ECochG) permet l'étude détaillée de la fonction périphérique, l'enregistrement en surface des réponses évoquées du tronc cérébral (RETC) permet l'évaluation de la conduction nerveuse jusqu'au niveau du colliculus. L'ensemble de ces réponses a été simultanément étudié chez des sujets pour lesquels une tumeur cérébrale affectant les voies auditives a été confirmée chirurgicalement par la suite. Des informations intéressantes ont pu être obtenues à partir de : 1) la comparaison des amplitudes des composantes sensorielles et nerveuses dans les réponses ECochG ; 2) la comparaison des seuils ECochG, RETC et audiométriques ; 3) la mesure de l'intervalle de temps séparant la réponse du nerf auditif (pic  $N_1$  à l'ECochG) et celle du colliculus (pic  $P_{1-4}$  des RETC) ; 4) les comparaisons ipsi et contralatérales. Les mêmes mesures effectuées dans les cas de pathologie purement cochléaire (principalement le syndrome de Ménière) ont montré des différences significatives.

## ZUSAMMENFASSUNG

### Transtympanale und Oberflächenableitungen in der Diagnose retrocochlearer Störungen

Eine Anzahl von Patienten mit später operativ erzielten intracerebralen Tumoren wurden mit Hilfe der transtympanalen Elektrocochléographie (ECochG) und durch Oberflächenableitungen von Brainstem-evoked Responses (BER) untersucht. Während die ECochG eine genaue Untersuchung der peripheren Funktionen ermöglicht, kann mit Hilfe der BER die Nervenleitung bis hinauf zum Colliculus Inferior geprüft werden. Dabei konnten wertvolle Informationen erhalten werden durch : 1) einen Amplitudenvergleich von sensorischen und neuronalen Komponenten des ECochG ; 2) einen Vergleich von ECochG, BER und Horschwelle ; 3) Messungen des Zeitintervalls zwischen Hornervantwort (ECochG-Komponente  $N_1$ ) und der Antwort des Colliculus Inferior (BER Welle  $P_{1-4}$ ) und 4) Seitenvergleiche. Entsprechende Messungen bei pathologischen Cochleabefunden, hauptsächlich bei Ménièrescher Krankheit, ergaben deutlich signifikante Unterschiede.

## REFERENCES

- Aran, J. M. 1973. Clinical measures of VIIIth nerve function. *Adv. Oto-Rhino-Laryngol.* 20: 374.
- Brackmann, D. E. & Selters, W. A. 1976. Electrocochléography in Ménière's disease and acoustic neuromas. In *Electrocochléography* (ed. R. J. Ruben, C. Elberling &

These filters were regulated for the following frequencies: low pass 140 Hz, and high pass, 1.6 kHz. The amplifier gain was maximal 1 e 5 volts. This amplifier was connected by a cable to the head-rest in the incubator.

(II) A modulator with a memory of 1074 words. The analysis time was 20 ms. It was equipped with an automatic artefact rejection system. It was possible to effect any number of passages between 1 and 9900. In general for evoked potentials 2048 stimuli were used.

(III) A stimulation control module to determine the number of clicks per second. In the cases observed the recurrence frequency was fixed at 20/s.

(IV) A trigger to synchronize analysis and sound stimulation.

(V) An oscilloscope

(VI) An X/Y recording table

(VII) An AX6 system for oscilloscope reading of recordings stored in the memory with the possibility of channel selection per matching inversion of the polarity of stimulus.

(VIII) A click generator (100  $\mu$ s)

(IX) A TDH 39 earphone equipped with a flex.

#### *The examination proper*

The examination requires particularly stringent conditions of asepsis. First the technical team was subject to the rules concerning entry into the Premature Unit: that is removal of overalls and shoes and donning of sterile overalls and slippers. A cap was also necessary. Masks were not compulsory. It was necessary to scrub up for 10 minutes.

The baby was brought into the examination room in its incubator. All the connections remained constant so that the conditions of humidity and temperature did not vary. The baby was brought in by the nurse in charge who remained present throughout the examination.

The examination was tuned to come at their changing and feeding. The babies slept throughout the examination and thus remained perfectly calm.

The electrodes used were of the electroencephalography needle type. They were inserted intradermally. The positive electrode was placed at the vertex. The differential electrode was placed on the mastoid of the ear being tested and the earth on the forehead. The impedance of these electrodes must not exceed 5000  $\Omega$ . Faulty impedance due to incorrect positioning of the electrodes greatly hinders correct examination. It leads to automatic artefact rejection according to the amplitude of the response. The electrodes were placed by the nurse.

The earphone was placed on the baby's ear. In 17 cases the left ear was examined and in 2 cases the right ear.

The parameters were then fixed: the pass band of the amplifier between 140 and 1600 Hz, and the gain 5  $\mu$ Volts. Analysis time was fixed at 20 ms, the recurrence interval at 20/s.

## RESULTS

The results are obviously incomplete. They are interpreted with great caution because the series is so short.

### *Pattern*

What is remarkable is that whatever the gestational age or the weight of the baby, the pattern clearly shows Jewitt's five waves provided the high intensity recording 90 or 80 decibels, was observed.

It is nevertheless important to note that if the baby was calm the J wave could be followed as far as 30 dB without any risk of error and in favourable conditions as far as 70 dB.

In this first study amplitude was ignored although it may be mentioned that it decreased in proportion to the intensity of the stimulus.

### *Latencies*

Three intensities: 80, 60 and 50 dB SPL were selected for the study of latencies. The latency of the J wave and the difference between AP and J were measured more specifically.

Since the examination was carried out in the

## A STUDY OF BRAIN STEM EVOKED RESPONSES IN PREMATURES

A Morgon and B Salle

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At the Centre d'Audiophonologie an approach to auditory function in premature has previously been attempted in two studies (i) one which examined the reactions of premature to auditory stimulations (ii) the other examined the stapelial reflex in prematures

In the light of these studies it appeared that as audition matures it progresses from lowpitched to high pitched sounds and from sounds of greater to those of lesser intensity In other words the more premature the baby the greater the necessity to use intense low pitched sounds in order to evoke a response

The use of electrophysiological techniques with prematures involves major difficulties with vertex potentials and considerable difficulties when it is necessary to attempt electrocochography

With the brain stem responses it is possible to examine auditory function in prematures provided certain essential conditions are fulfilled Prematures are extremely delicate and are particularly susceptible to infection

In addition to the physiological value of this examination there is a therapeutic consideration It is sometimes absolutely necessary to use an ototoxic drug in the treatment of a premature and its toxic effects on the cochlea can be monitored by means of BSRA

phonologie In this Unit the prematures are maintained under computerized supervision. For examination purposes an electrophysiological unit has been installed

The difficulties involved in this type of experiment account for the fact that the number of cases is rather limited

### *The population*

Fifteen cases representing 19 recordings were retained The pathological cases are excluded Two of them are quoted as an example in addition to these statistics None of the children involved in the study presented any pathological reaction of any kind

All the babies were in incubators They were not selected The recording team arrived on a specified day The Unit supervisor indicated a normal premature available for examination An effort was made to examine very prematures i.e. those with a low gestational age and low weight

Three of the 15 prematures were examined more than once 2 of them twice and one three times

The age range of these prematures varied from 30 to 42 weeks (g.a.) The weight range was from 1 100 g to 2 050 g The time between admission to hospital and examination varied from 55 days to 1 day

### PROCEDURE

This study was performed in the recently opened Premature Unit of the Hôpital Edouard Herriot Lyon The Unit is part of the same hospital complex as the Centre d'Audi-

### *Equipment*

The equipment used for recording brain stem in prematures was MEDELEC manufactured by RACIA It consists of (1) A modulator-amplified and a pre-amplifier with filters

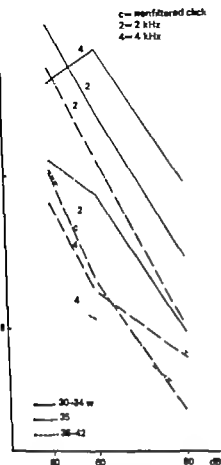


Fig 1 Latencies  $J$  as function of gestational age

*Respective influence of age and weight*  $J$  wave latency for non-filtered clicks was compared in the four conditions where the variables were as follows: gestational age alone; weight alone; weight and age in two groups; weight and age in three groups.

Gestational age proved to be the most important factor, but the influence of weight was greater for the lower weights and with lower intensity stimulation, that is 60 and better still 70 dB.

#### *Little opportunity to observe pathological recordings*

Our experience was limited to one case of a full-term baby weighing 3300 g admitted to hospital with pyocyanic septicaemia treated with amikacin. This baby died. The B S R.A.

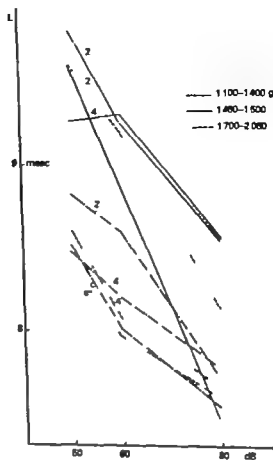


Fig 2 Latencies  $J$  as function of weight.

recording performed a week before death was completely flat.

#### *A single extended study in ideal conditions of one baby examined three times*

1200 g, 35th week  
1500 g, 38th week  
2050 g, 42nd week

The quality of the recordings made it possible to measure the latencies of  $J$ ,  $J_{N1}$ ,  $J$  and  $J_{N1}$  waves. Latency at 2050 g reached at the 42nd week, full-term, was not the same as for an adult but AP- $J_N$  was close to it.

#### CRITIQUE

The major criticism of the present study is the limited number of observations made. In fact



Table I

| Name          | Date of birth | Gest. age (weeks) | Weight (g) | Date of exam. | Gest. age (weeks) | Weight (g) | Duration of hosp. and weight |
|---------------|---------------|-------------------|------------|---------------|-------------------|------------|------------------------------|
| Rémi B        | 09 04 79      | 33                | 1 390      | 20 04 79      | 35                | 1 350      | 09.04-18.05/1 960            |
| Hayat M       | 13 03 79      | 32                | 900        | 19 04 79      | 37                | 1 700      | 13.03-10.05/2 090            |
| Cyril J       | 10 05 79      | 34                | 1 640      | 17 05 79      | 35                | 1 500      | 10.05-11.06/2 100            |
| Laurence L. 1 | 29 05 79      | 29                | 780        | 13 07 79      | 35                | 1 700      | -                            |
| Laurence L.   | -             | -                 | -          | 25 07 79      | 37                | 1 450      | -                            |
| Sabine V      | 31 05 79      | 30                | 1 450      | 31 05 79      | 30                | 1 450      | -                            |
| Benjamin B. 1 | 28 04 79      | 30                | 1 550      | 10 05 79      | 31                | 1 400      | 28.04-02.07/2 460            |
| Benjamin B.   | -             | -                 | -          | 31 05 79      | 34                | 1 500      | -                            |
| Nelly N       | 20 01 79      | 30                | 1 000      | 23 02 79      | 35                | 1 300      | 20.01-11.04/1 120            |
| Chrystelle M  | 06.04 79      | 32                | 1 050      | 11 05 79      | 37                | 1 330      | 06.04-11.07/2 370            |
| Adrienne C. 1 | 05 03 79      | 33                | 1 170      | 23 03 79      | 35                | 1 100      | 05.03-04.05/2 170            |
| Adrienne C.   | -             | -                 | -          | 17 04 79      | 38                | 1 500      | -                            |
| Adrienne C. 3 | -             | -                 | -          | 03 05 79      | 47                | 050        | -                            |

absence of any pathological phenomenon the two criteria likely to have any influence were gestational age and weight

**Influence of gestational age** The recordings were divided into three groups (1) 30-34 weeks gestational age (2) 35 weeks (3) 36-42 weeks

The figures obtained were significant for both AP-H and J latency. As hearing matured the latency of the J wave diminished. It should be noted that the J<sub>v</sub> wave for non-filtered clicks and the J<sub>v</sub> wave at 4 000 behaved in a similar fashion whereas latency remained high at 2 kHz.

The decrease in AP-J<sub>v</sub> was not linked exclusively to the shortening of J<sub>v</sub> latency but also to modification of the J<sub>i</sub> latency.

**Influence of weight** The observations were divided into three groups according to weight 1 100-1 400 g 1 450-1 500 g 1 700-2 050 g

The results were not at all significant since those in the lowest weight category produced results which were almost identical with those of the group with the highest weight for non-filtered clicks at 4 kHz. Nevertheless the influence of weight can be ascertained.

First the cases were divided into two groups (a) babies aged between 30 to 35 weeks and weighing less than 1 450 g and (b) babies aged between 36 to 47 weeks and weighing more than 1 450 g. The results were significant only for non-filtered clicks and 4 kHz.

Second then the cases were divided into three groups (a) babies aged between 30 to 34 weeks and weighing less than 1 500 g (b) babies aged 35 weeks and weighing less than 1 500 g and (c) babies aged between 36 to 42 weeks and weighing more than 1 450 g. The results were then significant.

Table II

| Name        | Date of birth | Gest. age (weeks) | Weight (g) | Date of exam. | Gest. age (weeks) | Weight (g) | Dur. of hosp. and weight |
|-------------|---------------|-------------------|------------|---------------|-------------------|------------|--------------------------|
| Pierre D    | 28 1 78       | 39                | 1 580      | 22.01 79      | 42                | 1 800      | 28 1 78-06.03 79/2 120   |
| Ahmed N     | 05 03 79      | 32                | 1 890      | 11 03 79      | 34                | 1 800      | 05 03 79-03 04 79/1 160  |
| Emilie N    | 19 02 79      | 32                | 1 820      | 26 07 79      | 33                | 1 820      | 19 02 79-12 03 79/2 120  |
| Angélique D | 13 03 79      | 33                | 1 770      | 06.04 79      | 35                | 1 700      | 13 03 79-19 04 79/2 110  |
| Véronique B | 7 1 78        | 37                | 1 100      | 01 02 79      | 39                | 1 700      | 08 1 78-19 07 79/1 750   |
| Alexis D    | 14 01 79      | 35                | 2 020      | 23 01 79      | 36                | 1 700      | 07 12 79-28 01 79/2 220  |
| Adrienne C  | 05 03 79      | 33                | 1 170      | 03 05 79      | 42                | 050        | 05 03 79-04 05 79/1 170  |

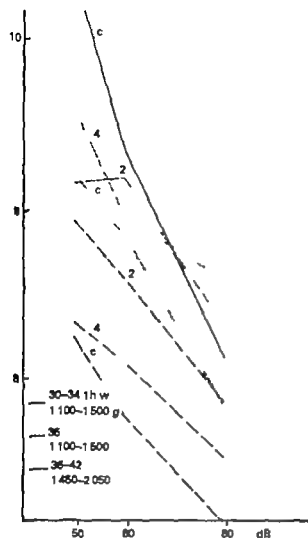
## DISCUSSION

*Scalise to Margon:* Did you use in your studies any frequencies other than 2 000 Hz and 4 000 Hz?

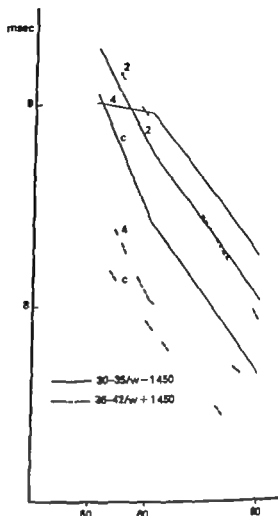
In our studies on 4 premature babies and 141 full-term neonates the best responses on application of ERA are obtained by using 900 Hz in prematures and 1 000 Hz in full-term neonates. There were only single responses on stimulating with 500 Hz. What is your comment on this?

*Margon (Reply):*

The choice of frequencies depends on two factors: (i) to keep the same procedure in baby, infant or adult, and (ii) to use frequencies to cover the larger part of the frequency field.



Figs 3-4 Latencies  $J_1$  in function of weight and gestational age



no measurement was made unless five results were obtained for a given intensity and frequency

The work continues. It is the subject of a thesis which will collate more informations and a greater number of observations either conforming or contradicting these first results.

Nevertheless in spite of the procedural difficulties the observations presented here were obtained in ideal conditions providing easy reading of the recordings.

### CONCLUSIONS

The study of BSRA in premature in cubators reveals the following four points

Auditory function matures as follows: non filtered clicks and the 4000 Hz frequency

develop in a parallel fashion. The 2000 frequency seems to be delayed.

The latency figures for the  $J_1$  wave diminish as hearing matures but the premature baby does not have the same latencies at term as the new-born baby.  $AP-J_1$  also diminishes as maturity nears principally because of the reduction in  $J_1$  latency but also to a certain extent because of the modifications in  $J_1$  latency which oddly enough seems to lengthen.

The most important element remains gestational age but the influence of weight can be totally eliminated.

BSRA appears to be a good method which to survey premature babies in the neonate of the incubator or/and with ototoxic treatment.

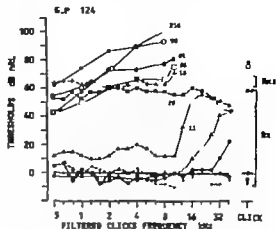
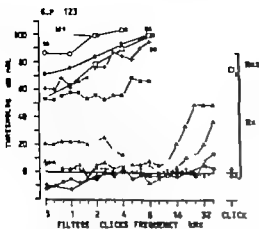


Fig. 1. Frequency threshold curves of the responses recorded at the round window for guinea pigs at various times indicated in days (small characters) after the beginning of the first amikacin treatment (day 0). Note the

rapid evolution during the first treatment (Rx1: 14 450 mg/kg/day) and the small changes during and after the second treatment (Rx2: 20 doses), particularly for GP 124 (right).

while the other ear not involved by the procedure, served as a control at time of sacrifice of the animal. The surgical procedure as well as the method of stimulating and recording from this electrode in awake animals have been described in detail elsewhere (Aran & Erre, 1979) and used in various studies (Aran & Darrouzet, 1975; Aran & Cazals, 1978). As usual the auditory nerve compound action potential (CAP) thresholds to filtered clicks from 0.5 to 40 kHz are determined (CAP audiograms) the intensity levels being referred to the mean threshold of normal responses (dB nHL). Although we normally use a Bruel & Kjaer condenser microphone cartridge (4134) directly coupled to the ear as a transducer in these experiments as soon as the thresholds became elevated we had to use a TDH 39 Telephonics earphone in order to obtain higher sound levels although the frequency of the transient stimuli could not then exceed 10 kHz. Responses to the unfiltered click using this earphone were also recorded from the very beginning of the experiments so that patterns of the responses and input-output amplitude and latency functions could be monitored over the entire experiment. As usual for the recordings we systematically used averaging

and clicks of alternate polarity to cancel out any microphonic potential.

#### Effects of the first treatment

These 5 implanted guinea pigs having normal click-evoked responses and CAP audiograms received a first treatment of amikacin identical with that used in our earlier studies (Aran et al. 1979; Cazals et al. 1979b) i.e. 14 intramuscular injections of amikacin (daily except during week-ends) at doses of 450 mg/kg. Responses were monitored every 2 to 4 days before and also during the treatment, then with longer time intervals between tests after them.

For all the guinea pigs CAP audiograms started to alter already at the end of the first week of treatment, beginning with an elevation of the high frequency thresholds (Fig. 1). By the end of the treatments all the CAP audiograms showed the same pattern with thresholds around 50–70 dB at 0.5 kHz and progressively increasing with the frequency up to 90–100 dB at 8 kHz.

Then shortly before or after the end of the treatment 2 guinea pigs died (at 14 and 20 days).

By this time the click-evoked responses were profoundly altered in all the animals

## ELECTROPHYSIOLOGICAL MONITORING OF THE COCHLEA DURING AND AFTER TOTAL DESTRUCTION OF THE ORGAN OF CORTI

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Anne Guilhaume and Jean Paul Erre

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**Abstract** A series of acoustically evoked potentials can be recorded from the cochlea up to the auditory cortex in guinea pigs where the organ of Corti has been totally destroyed after extensive treatment with amikacin, but where some of the spiral ganglion neurons always remain and where the vestibular receptors are only slightly affected. The cochlear responses have been monitored in guinea pigs permanently implanted with a round window electrode and receiving such treatment. The normal auditory nerve compound action potential disappears within a few days, while the very typical response (biphasic short latency (0.3 ms) small amplitude) appears. This response then remains remarkably constant in time as far as we could observe (up to almost one year). This response might be a component of the normal response undiscernible under normal conditions but revealed by the selective impairment of the labyrinth by amikacin. In contrast to the global effect of every other known otodestructive agent. Some basic questions still remain: which fibres are effectively stimulated (cochlear or vestibular)? what are their central projections and what kind of sensation is conceivably associated with these responses?

Clear click-evoked electric responses have been recorded at the round window of guinea pigs cochleas which about one month after intense treatment with the aminoglycoside antibiotic amikacin presented a total loss of inner and outer hair cells along the entire length of the basilar membrane except for a very few remaining outer hair cells in the third row at the extreme apex while the vestibular function and receptors seemed to be preserved and some nerve fibres survived in the remnants of organ of Corti (Aran et al 1979).

In subsequent experiments identical results have been obtained in the same conditions and the effects of phase of the stimulus, anoxia and masking on such responses have strongly suggested that they were of a neural

nature (Cazals et al 1979b). These responses are followed by an activation of the central nervous system and of the auditory cortex as evidenced in recordings from surface electrodes and from electrodes on the auditory cortex. However the brainstem recordings show an absence of waves IV-V complex and cortical responses although specifically limited to the auditory cortex are delayed by about 4 ms as compared with the click-evoked responses in the normal guinea pig (19 instead of 15 ms) (Cazals et al 1979a) (see an example in (Fig. 5)).

In order to obtain more information about the nature of the early cochlear responses, their onset and evolution in time during and after (up to one year) the same—or more intense—amikacin treatment have been studied either together or separately on the same or different guinea pigs. For this purpose we used the method of chronic recordings of cochlear responses in awake animals complemented by acute recordings from both ears immediately before sacrifice. We report here the results of various experiments in which unless otherwise specified the times of observation are referred always to the first day of treatment for each animal (day 0).

### *Short term Evolution during Amikacin Treatments*

#### *Methods*

Five normal pigmented guinea pigs (GP) were equipped with an electrode permanently implanted on the round window of the left ear

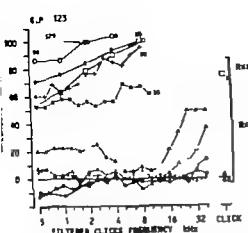
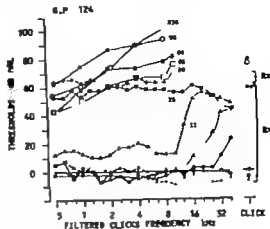


Fig. 1. Frequency threshold curves of the responses recorded at the round window for guinea pigs at various times indicated in days (small characters) after the beginning of the first aminoglycoside treatment (day 0). Note the



rapid evolution during the first treatment (Rx1: 14 450 mg/kg/day) and the small changes during and after the second treatment (Rx2: 20 doses) particularly for GP 124 (right).

while the other ear, not involved by the procedure, served as a control at time of sacrifice of the animal. The surgical procedure as well as the method of stimulating and recording from this electrode in awake animals have been described in detail elsewhere (Aran & Erre 1979) and used in various studies (Aran & Darrouzet 1975; Aran & Cazals 1978). As usual, the auditory nerve compound action potential (CAP) thresholds to filtered clicks from 0.5 to 40 kHz are determined (CAP audiograms); the intensity levels being referred to the mean threshold of normal responses (dB nHL). Although we normally use a Bruel & Kjaer condenser microphone cartridge (4134) directly coupled to the ear as a transducer in these experiments as soon as the thresholds became elevated we had to use a TDH 39 Telephonics earphone in order to obtain higher sound levels, although the frequency of the transient stimuli could not then exceed 10 kHz. Responses to the unfiltered click using this earphone were also recorded from the very beginning of the experiments so that patterns of the responses and input-output amplitude and latency functions could be monitored over the entire experiment. As usual for the recordings we systematically used averaging

and clicks of alternate polarity to cancel out any microphonic potential.

#### Effects of the first treatment

These 5 implanted guinea pigs, having normal click-evoked responses and CAP audiograms, received a first treatment of amikacin identical with that used in our earlier studies (Aran et al 1979; Cazals et al 1979b), i.e. 14 intramuscular injections of amikacin (daily except during week-ends) at doses of 450 mg/kg. Responses were monitored every 2 to 4 days before and also during the treatment, then with longer time intervals between tests after them.

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Then shortly before or after the end of the treatment 2 guinea pigs died (at 14 and 20 days).

By this time the click-evoked responses were profoundly altered in all the animals.

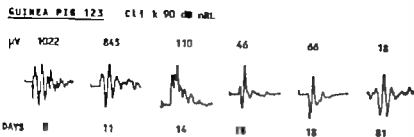


Fig. 2 Patterns of averaged click-evoked responses at the round window (using clicks of alternate polarity to cancel out any macrophonic potential) at various times for GP 123. The numbers above the traces indicate the peak-to-peak amplitude in microvolts of the corresponding response. The last trace on the right (day 81) is that recorded at the end of the 2nd treatment (see Fig. 1). Trace duration, 5 ms; click at the tympanic membrane 0.9 ms after the beginning of the traces (arrow). Note the important positive component which disappears from day 16 to day 18.

showing the typical characteristics already observed in the earlier experiments about one month post Rx: i.e. high thresholds (50 to 70 dB nRL), very short latency (0.3 ms) and amplitude of the order of 100  $\mu$ V at 100 dB which, although 10 times smaller than in the normal, gives very clearly discernible responses even without the use of averaging (Figs 2 and 3). However, for one guinea pig (GP 124) the responses were less affected and these characteristics were established only during a second, more extensive treatment (Fig. 3).

#### Effects of a second, longer treatment

In the 3 remaining guinea pigs (GPs 117, 123 and 124) a second treatment, using the same daily dose, was administered but over 20 injections (4 weeks) instead of 14, starting at day 56 (that is 38 days after the end of the first treatment). The responses were similarly monitored in 2 guinea pigs (GPs 123 and 124). Electrode failure prevented such monitoring in the third one (GP 117), though it could be used for the long-term study by testing the right ear at time of sacrifice (at day 216), as revealed later.

During this second treatment there was a slight elevation of the thresholds (Fig. 1) but the waveforms were not notably altered for GP 123, while they progressively reached the typical pattern for GP 124, with the complete disappearance of an early positive component

(summing potential?) clearly observed usually during the treatment (Figs 2 and 3).

#### Long-term evolution

By this time, more than one month after the end of the first treatment—and particularly after the second one—we might say in view of the histological results in the earlier experiments (Aran et al. 1979; Cazals et al. 1979b) that the organs of Corti had been totally destroyed. The constancy in time of such paradoxical responses acoustically evoked without organ of Corti have been further evaluated in guinea pigs from two different experiments: in the 3 guinea pigs mentioned above (GPs 117, 123 and 124) and in 3 guinea pigs from our earlier experiments (GPs 189, 195 and 196) (Cazals et al. 1979b) which were not initially implanted and which had received only one series of 14 injections. Responses were monitored in one of them (GP 189) implanted about 2 months after the end of the treatment (at day 85) until day 347, while the 2 other, not implanted, were sacrificed at day 262 just after acute recordings.

GP 124 showed remarkably constant responses until the sacrifice, more than 7 months later (at day 216) (Figs 1 and 3). However, for GP 123 the progressive threshold elevation continued after the end of the second treatment until no response was recordable at 4½ months (Fig. 1). At time of sacrifice (>7 months) responses could be recorded from

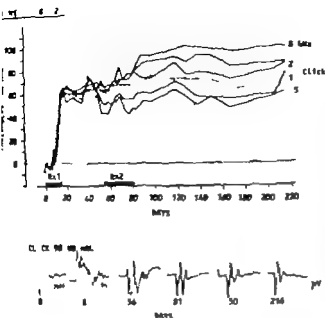


Fig 3 Upper curves Evolution of the thresholds to the various filtered clicks (from 0.5 to 8 kHz) and to the click (---) during the entire experiment including the aminocaproic treatments (Rx1 and Rx2). Note the relatively good low frequency thresholds. Lower trace Click-evoked responses from the same guinea pig at various times. Traces as in Fig. 2. Peak-to-peak amplitudes indicated in microvolts in small characters. Note the disappearance of the early positive peaks during the second treatment (days 36 to 81) It is not obvious whether the final response (at days 180 or 216) is present or not in the responses at 0, 18 or 36 days.

the ears and from the auditory cortices (with contralateral acoustic stimulation) in GPs 124 and 117 (Figs. 4 and 5) while no response was recordable either from ears or from the auditory cortices of GP 123.

In GP 189 responses were remarkably constant both in pattern and amplitude from day 0 (implantation) to day 347 (sacrifice) (Fig. 6). Similar responses could be recorded in GP 196

(Fig. 7) while no response could be recorded from either the left and right ear of GP 195. However for this GP it was obvious either during electrode implantation for acute recordings or later when preparing the ears for histology that it had been suffering from bilateral otitis media (thickening of bone and presence of membranous material in the middle ears)

#### GUINEA PIG 117

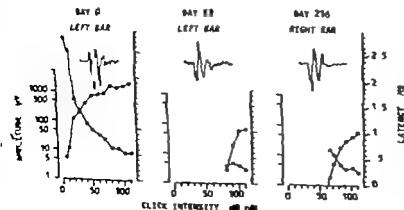


Fig 4 Patterns of the click-evoked responses at 90 dB nHL (upper trace) and input-output amplitude and latency functions at days 0 and 32 from the left ear and at day 216 from the right ear of GP 117 which received the same treatment (Fig. 3) as GPs 123 and 124. Traces as in Fig. 2.



## GUINEA PIG 124

DAY 216

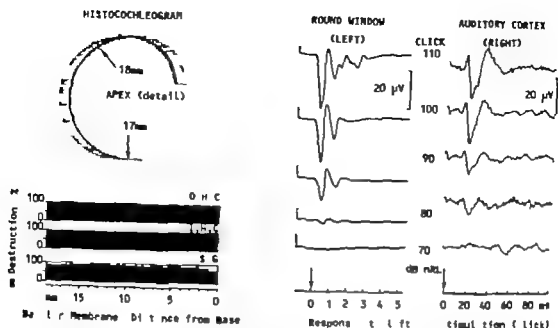


Fig 5 Histocochleogram of the left ear and responses to left ear stimulation from the round window of the left ear and from the right auditory cortex recorded the day of sacrifice (day 216) for GP 124 (see Figs 1 and 3). Note the only remaining hair cells (●) displayed in the detailed reconstruction of the apex (each 10 µm section has been inspected and represented). Arrows with numbered

millimetres indicate distance from the oval window. Note the total inner hair cell (IHC) loss and the almost total outer hair cell (OHC) loss and the small proportion of remaining spiral ganglion neurons (SG) along the basilar membrane. Note the good correlation between round window and auditory cortex responses. Arrival of the click at the ear (time 0 (arrow)).

## Histology

As in the former studies again no hair cells inner or outer could be detected along the entire length of the basilar membrane in any of the guinea pigs except for very few outer

hair cells of the third row at the extreme apex. In several guinea pigs we used a serial section technique and by sectioning the entire cochlea in 10 µm steps each 10 µm section was inspected so that no hair cell could be missed

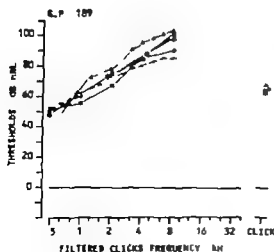


Fig 6 Frequency threshold curves and responses to the click from day 85 to day 347 for GP 189 which received only one treatment of amikacin (14×450 mg/kg/day)

Traces as in Figs 1 and 4. Numbers in small characters indicate the peak-to-peak amplitude of the responses in microvolts.

16 196

GUND I BOW RESPN ES SA 262

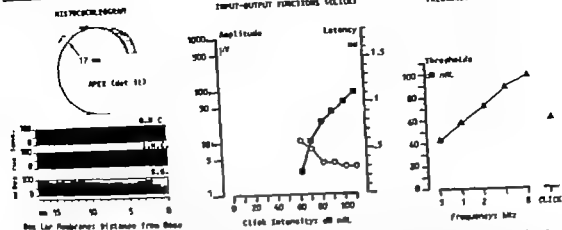


Fig 7 Hysterochleogram (as in Fig 5), of GP 196 which received the same single treatment as GP 189 but was sacrificed at day 262 instead of 347 and input-output amplitude and latency functions (click stimulation) and frequency threshold curve of the responses recorded from the round window the same day. This guinea pig typifies the cochlear condition and types of response obtained after such treatment.

frequency threshold curve of the responses recorded from the round window the same day. This guinea pig typifies the cochlear condition and types of response obtained after such treatment.

Then no more than a dozen outer hair cells could be counted. Examples are shown in Figs. 5 and 7. When counting the spiral ganglion neurons as well there appeared to be a significant loss, more pronounced at the apex than at the base and also more pronounced after the two treatments than after only one, for the same delay between treatment and sacrifice ( $>7$  months). Also the loss seems more serious after 7 months than after 2, indicating a slow progressive neural degeneration. However in any case some spiral ganglion neurons still remain and as seen in electron microscopy a few nerve fibres running in the epithelial layer which covers the basilar membrane to varying extents. Also the vestibular receptors always appear much less affected. The condition of the ears of GPs 143 and 195 where no response could be recorded is comparable to the others.

## DISCUSSION

Two main items of information about these paradoxical peripheral responses acoustically evoked in the absence of the organ of Corti

are given by the short and long-term studies respectively.

These responses appear as soon as the normal compound action potential disappears. For all the guinea pigs this occurs within a very short transient period (threshold shift of about 60 dB within one week). At this time it is indeed difficult to understand the recorded patterns which obviously contain a relatively large proportion of positive d.c. components like the summing potential disappearing progressively. Then the typical response such as that observed in our earlier experiments at about 1–2 months after the end of the treatment is clearly established. Although this response would not be discernible in the normal response before treatment due to the too large amplitude ratio it might be possible to detect it in the distorted, smaller patterns during the transient period (Figs. 2 and 3).

The response characteristics are impressively stable over several months (Fig. 6). In contrast, during our earlier study over 7 months using kanamycin with similar daily doses but a shorter treatment (8 doses) a close parallel between progressive hair cell loss (as supported by many histological studies partic

ularly that of Gonzalez et al 1972) and a progressive change of the responses could be demonstrated. In the present condition using amikacin more extensively (14 doses) the hair cells disappear completely and quickly as early as one month post treatment (Aran et al 1979; Cazals et al 1979b) possibly earlier. There is certainly a progressive spiral ganglion degeneration which however is difficult to correlate with the evolution of the responses. Admittedly there are 2 guinea pigs (GPs 123 and 195) without responses but for one (GP 195) it could be ascertained that there was an added conduction loss. For GP 123 we do not have any such evidence but it is the only one in our series of experiments which could confirm the other data although a conductive impairment in this case cannot be ruled out.

It must be recalled that these responses seem to be effective for the activation of the central nervous system and particularly the auditory cortex. In effect whenever the auditory cortex responses were investigated in these and other experiments the results always correlated very well (thresholds) with that obtained at the periphery (Cazals et al 1979a).

These two observations, rapid onset and long term stability, indicate that the peripheral responses might originate from an already existing system in contrast to the slow neuro-morphological changes which occur after such drastic impairment of the cochlear structures (Spoendlin 1975). It is likely that this system lies in the vestibular receptors such as the saccule which as already mentioned is only slightly affected. However the vestibular receptors are also preserved during other treatments (e.g. kanamycin). Moreover on the basis of the present data it cannot be assessed whether the structure responsible for these responses is already functioning in the normal or does so only in such a clear-cut pathological condition.

Thus there still remain some basic questions some of which should be answered

soon: (i) Which nerve fibres are effectively stimulated and responsible for these responses (cochlear or vestibular fibres)? (ii) What are their exact central projections? (iii) finally what kind of sensation if any is evoked in such conditions?

## ACKNOWLEDGEMENT

This work was supported by INSERM grant no. 8-ASR-4 (Oreille interne).

## RÉSUMÉ

Des réponses évoquées par des stimulations acoustiques peuvent être enregistrées, depuis la cochlée jusqu'au cortex auditif chez des cobayes dont les organes de Corti ont été entièrement détruits lors de traitements intensifs par l'Amikacine mais où subsistent des neurones du plexus spiral et où les récepteurs vestibulaires semblent peu affectés. Les réponses cochléaires ont été suivies chez des cobayes implantés d'une électrode à demeure sur la fenêtre ronde et recevant de tels traitements. Le potentiel d'action composite normal du nerf auditif disparaît en quelques jours alors qu'apparaît cette réponse caractéristique (diphase, de latence courte (0.3 ms), de faible amplitude). Cette réponse demeure alors remarquablement constante dans le temps aussi longtemps que nous avons pu observer (presque un an). Cette réponse pourrait être un élément de la réponse normale masqué par celle-ci dans les conditions normales, mais démasqué lors de l'atteinte sélective du labyrinthe due au traitement d'Amikacine par opposition à l'action globale de tout autre agent ototoxique connu. Cependant quelques questions élémentaires sont toujours posées: quelles sont les fibres effectivement stimulées (cochléaires ou vestibulaires) quelles en sont les projections centrales et quelle sorte de sensation pourrait être associée à ces réponses.

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## DISCUSSION

**Speculation by Aron.** In some of your animals you recorded acoustic potentials in spite of an apparently complete loss of hair cells and spiral ganglion cells. This is in fact very surprising and can only be explained by an acoustic activation of some unaffected vestibular elements. I would also question any correlation between the ECOG response and functioning cochlear neurons. The quantitative morphological evaluation of the cochlea is of course crucial for the interpretation of these results. Such quantitative critical evaluation is probably very difficult on the basis of serial sections and I think combined surface and EM technique would be better. In our experience it can easily happen that one finds distorted rudimentary hair cells in EM preparations of cochleas: here in LM all hair cells seem to have disappeared.

**Barnes to Aron.** It is very difficult to find an explanation for the described observations. If we assume that all hair

cells are destroyed and not merely damaged and still functioning, we have to think of vestibular transformation. This vestibular source of the potentials seems to be very improbable—otherwise we would find some potentials more frequently in completely deaf patients. One hypothetical explanation seems to be a sinister phenomenon we all know named electrophony. As it is based on electromechanical transformation it could also be possible that mechanoelectrical transformation produces an electrical stimulation of the nerve fibres.

**Gilson to Aron.** I ask whether any adaptation studies have been performed. This would distinguish between a receptor potential or an artificial microphonic and a truly neural component.

**Beagley to Aron.** This response should perhaps be called 'hair-cell loss acoustic response' rather than a cellless response. Presumably the other cells of the organ of Corti are present?

**BM Johnstone** has postulated direct mechanical-stimulatory effect on the nerve fibres supplying the IHCs. It is possible that this is the origin of the response in the auditory nerve that Aron has described.

As part of the response has a short latency 0.3 ms, it suggests that some form of receptor potential is involved. It would be interesting to do d.c. recordings of the scala media to see if there is an endolymphatic d.c. potential present in the ears damaged with Amikacin.

**Bauer to Aron.** It would be interesting to know the frequency spectrum of the click stimuli. Assuming that it is response of the saccule, strong click may cause sudden dislocation of the stapes which might be a proper stimulus for the saccule. If this is so have you observed any movements of the eyes by nystagmography?

**Aron (Portmann repl.)**

Thank you for your comment. In the absence of Dr Aron I will try to do my best and invite you to our laboratory to see exactly what Dr Aron and his group do.

They carefully observed with light and electron-microscopy and they believe that the hair cells are destroyed.

The behaviour of the response proves that it is not an artefact. I thank the speakers for the ideas they gave to try to explain the phenomenon.

CLINICAL AND EXPERIMENTAL STUDIES  
ON THE INCORPORATION OF MATERIALS APPLIED  
AS COVERING FOR THE LABYRINTH WINDOW

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**Abstract** From among the materials used for covering the labyrinth window of the foreign materials gelfoam proved ideal from clinical and experimental points of view while of the materials of interstitial origin fascia, perichondrium and periosteum seem to be suitable for covering the labyrinth window resp for transplantations in the tympanic cavity

Otosclerosis is one of the forms of conductive hypacusia that can be best corrected In 1956 Shea introduced stapedectomy since when hundreds of thousands of otosclerotics have regained their hearing thanks to surgical operation

Otologists use various materials for stapedectomy Some operating otologists do not use foreign material in the tympanic cavity In this way for instance Portmann uses the stalk of the stapes to replace the removed stapes Bauer forms a cortical columella replacing the removed stalks with that

Some operating ear surgeons use plastic teflon prostheses for replacing the stalk of stapes Most of them—Schuknecht (1963) House (1970) and also we ourselves (Ribari 1976)—apply rustproof metal This rustproof metal may be steel tantalum or molybdenum as well For covering the oval window or the round window after rupture various materials are used for instance gelfoam perichondrium periosteum vein fascia fat and possibly blood (Goodhill 1971)

The results of operations carried out with different techniques generally bring about a 90% lasting result

At the Oto-Rhino-Laryngological Depart

ment of the University Medical School in Szeged we have carried out comparative investigations partly on the clinical patient material and partly by animal experiments to see how the result of the operation is affected by covering the labyrinth window with different materials and how the implants are incorporated into the middle ear

In our operations at first venous wall was used with polyethylene tube which gave rise to some problems fistulization and development of granulation in the vicinity of the venous wall More recently we have covered the labyrinth window with gelfoam or else used temporalis fascia in some cases

Of late we have almost always applied a wire prosthesis in our operations In the case of an obliterative juvenile otosclerosis a piston was inserted In case of applying a vein the closure of the post-operative airborne gap follows relatively slow The closure is often not even full

In Fig 1 a picture of a venous wall is shown removed from the oval window in connection with a reoperation The venous structure can be identified but a very strong rebuilding—a considerable fibrosis—can be observed too

The operation was performed 18 years ago The ossification of the removed vena can be observed in the interpositum The implanted veins were to be removed in more than one case It was proved by the operations that the venous wall formed a comparatively thick interstitial material the vibration of the thick

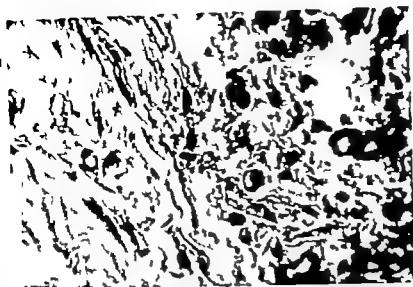


Fig 1

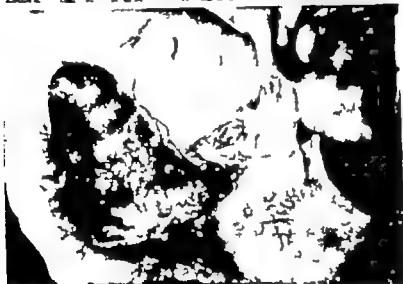


Fig 2

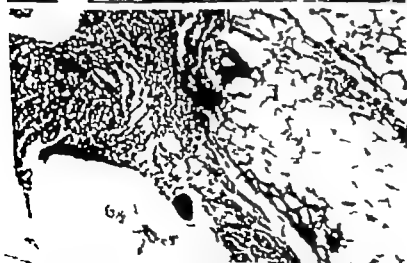


Fig 3

material was not satisfactory and at the same time a perforation could also develop on the implanted venous wall. The insertion andhesion of the vein was not satisfactory.

We have carried out comparative investigations on 20 patients in our clinical patient material. On one side the oval window was covered with gelfoam, on the other side fascia was applied to cover the oval window. One year later it was found by postoperative audiometry that in the case of a gelfoam-wire prosthesis the air-bone gap closes much faster than in cases where fascia alone is applied.

One year after the operation was performed no gelfoam was found in the patient material nor any difference in the fasciae. Thus we see that the late results were not affected by the difference between the two implanted materials.

When applying gelfoam we have not experienced a single case of toxic damage to the inner ear. Gelfoam in our experience never behaves like foreign matter.

Although we know that the conclusions drawn from animal experiments are not valid in every case in respect of man, we have investigated in experiments made on guinea pigs how the incorporation of the materials used for covering the labyrinth windows takes place.

At first we carried out the operation on guinea pigs under urethane anaesthesia by freeing the bony wall of the bulla ossea and then, after removing the stapes, covering the oval window with different materials: gelfoam, fascia, perichondrium, perosteum, resp. fat and closure of the wound.

Of late we have already performed the operation via the auditory canal after deflecting the tympanic membrane. The animals were decapitated at different times: one 4-6 weeks resp. 2 months after the operation, and the cochleae were removed together with the materials put on the labyrinth windows.

It can be seen even by macroscopic inspection that gelfoam, perosteum and other con-

nective tissue materials soon become incorporated in a majority of cases. It can be seen in the figure that the incorporation of blood vessels begins very soon as well (Fig. 2). Only in the case of fat transplantation have we observed frequent necrotizing of the fatty tissue and the development of otitis in animals. This did not occur (or only very rarely) when other materials were applied. In most cases, the implant and even the fatty tissue incorporated very well.

The material removed was fixed in formalin, decalcified, stained and histologically examined. The histological investigations also showed that the histological materials used were incorporated into the middle ear very well.

Gelfoam shows an histological transformation already a week after implantation and the fascia also incorporates well one week after the operation. In the place of gelfoam, connective tissue develops within a fortnight and the gradual absorption of gelfoam is visible. The perosteum very soon adheres to the environment of the bone, filling the window with a fine membrane. The response of fatty tissue can be observed in Fig. 3.

At the edge of the fatty tissue granular tissue develops 6 weeks after the operation, pressing forward somewhat towards the inner ear. The same is discernible still better when magnified strongly. From the clinical results presented and the other histological investigations as well, we have drawn the conclusion that as opposed to the histological interposition, gelfoam is well suited for application to close the oval window. The acoustic results in the case of applying gelfoam are also best in the early period.

The advantage of gelfoam is that it is easily handled, is plastic and closes the window very well. The difference ceases to exist according to the clinical data after the incorporation of the tissue. Of the interstitial materials, perosteum and fascia can be applied very well to cover the oval window. On the other hand, the venous wall and fatty tis-

me proved to be a less good interpositum. The mucous wall partly calcifies, is transformed and becomes stiff. A part of the fatty tissue necrotizes, another part forms in some of the cases, undesirable granular tissue.

Before transplanting the materials we had attempted application of a low-energy helium-neon laser in order to observe how the irradiation of the low-energy laser affects the adhesion of transplantation. Unexpectedly the low-energy laser promotes the vascularization and adhesion of transplantation considerably. As a result of irradiation the transplant incorporates faster and vascularization also follows earlier.

We have also applied irradiation to close the perforation. The low-energy laser induced rapid epithelialization of the perforation.

We have applied irradiation to close experimental tympanic membrane perforations as well. The low-energy laser induced rapid epithelialization of the perforation. It was shown by the histological investigation carried out after the low-energy laser irradiation that no change arose. On the other hand oedema was formed at elevated energy levels.

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## DISCUSSION

Portman to Ribár. I think that we have to avoid gelfoam for closure of the oval window. In 1964 I did 300 cases with gelfoam and the sensorineural hearing loss went up from 0.7% to more than 6%.

I think that we must recommend absolute avoidance of gelfoam—if it is not toxic by itself, which is not proved, it does result in this membrane which does not protect the labyrinth sufficiently against inflammation from pharynx and tubal region.

Reference to Ribár. Tissues acquire independence in late life as an embryonic period.

Ribár (Reply)

Thank you very much for your questions.

The gelfoam made a very thin membrane but it has no toxic effect.

It depends on the method of sterilization.

An ideal material is fusible with perforation. Fat and vein are no good.



DEVELOPMENT OF ACETYLCHOLINESTERASE (AChE) STAINING  
IN HUMAN FETAL AUDITORY CORTEX

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**Abstract** In this study acetylcholinesterase (AChE) staining methods have been used to demonstrate the development of the prospective afferent fibres of auditory cortex in human fetuses ranging between 8 and 28 weeks of gestation. Earliest AChE positive staining was found in fetuses at 20-4 weeks in the neuropil of the marginal zone and throughout subplate layer of the auditory cortex. On the basis of this characteristic AChE staining pattern the auditory cortex may be delineated from surrounding cortical areas. At 24-26 weeks during intensive lamination of the cortical plate AChE-positive reaction appears in the deep part of the cortical plate. In the oldest fetuses (28 weeks) a columnar pattern of vertical darkly stained zones was seen in the middle third of the cortical plate. We conclude that AChE staining is characteristically distributed within cytoarchitectonic compartments and synaptic layers of the developing auditory cortex. The AChE-positive layers coincide with the laminar pattern of synaptogenesis. Thus AChE positive reaction during development may serve as a parameter of cortical afferent (thalamic?) innervation of the auditory cortex. In addition AChE reactivity may indicate the specific transmitter nature of the afferent fibres in the auditory cortex.

(Vaughan 1975) it is necessary to determine afferent input to the developing cortical layers.

The most promising morphological approach to the analysis of afferent cortical input in the human fetal cortex is to demonstrate ingrowing fibres by histochemical techniques (Kostović Kelović Krmpotić Nemančić & Kračun 1979 Kostović 1979) and to determine their prospective transmitter properties. In the present study histochemical methods have been used to determine the spatial and temporal pattern of the development of the acetylcholinesterase (AChE) positive staining in the auditory cortex of the human fetus. The AChE reaction is used primarily for demonstration of fibre ingrowth (Kostović Knežević Kostović Krmpotić Nemančić & Kelović 1979) and the pattern of cortical organization (Kostović 1979). In addition we plan to obtain some indicators in the prospective cholinergic innervation of the auditory cortex.

Our previous morphological observations (Krmpotić Nemančić Kostović Nemančić & Kelović 1979) suggested that circuitry elements (dendrites axons synapses) are present in the human auditory cortex in an early phase of prenatal development and have consistent spatial relationship to the cytoarchitectonic layers. A similar pattern was also demonstrated on other sensory cortices in man and primates (Molliver Kostović & Van der Loos 1973 Kostović & Molliver 1974 Purpura 1975 Kostović Knežević Kostović Krmpotić Nemančić Kelović & Vuković 1978). In the light of the possible functional status of the early sensory circuitry in the human fetus

## MATERIAL AND METHODS

Brains of human fetuses ranging between 30-270 mm crown-rump length (8-28 weeks of gestation) were fixed for 48 hours by immersion in 0.1 M sodium phosphate buffer containing 1.5% glutaraldehyde plus 2% paraformaldehyde. For histochemical analysis the blocks containing temporal cortex were serially sectioned on a freezing microtome. Free sections were stained for AChE reaction with Lewis modification of Koelle's method (Krn



Fig 1 Low-power micrograph of the superior temporal cortex AChE staining. Human fetus, 22 weeks. Note boundaries of the prospective auditory cortex (double row) and approaching thalamocortical fibres (arrows). CP = cortical plate; SP = subplate layer; P = putamen; CI = capsula interna; CE = capsula externa.

jević & Silver 1966) and Karnovsky Roots method (Broderson Westrum & Sutton 1974). The specificity of AChE reaction (specific or non-specific AChE) was controlled by various inhibition tests. For cytoarchitectonic analysis adjacent sections were stained by the Nissl method. In addition we have used serially sectioned celloidin-embedded brains from the neuroembryological collection of the Department of Anatomy, Medical Faculty, Zagreb.

## RESULTS

In the youngest fetuses (8–20 weeks of gestation) the developing cortical layers (marginal zone, cortical plate and subplate layer—for definition of the cytoarchitectonic pattern see Krmpotić Nemanjić, Kostović, Nemanjić & Kelović 1979) were free of AChE staining. However, in the underlying intermediate zone the AChE-positive fibres were seen within capsula interna and capsula externa as early as 1 week of gestation. In the next period (20–4 weeks) during intensive lamination of the cortical plate, AChE staining of the neuropil was observed in two strata of the temporal cortex: marginal zone and subplate layer (Figs 1 and 2). In the deepest part of the cor-

tical plate we have seen only diffuse AChE staining (Fig. 2 asterisk). On the basis of the strong AChE staining in the subplate layer, the auditory cortex may be delineated from the surrounding temporal cortex which shows very weak AChE reaction (Figs 1 and 2). The AChE-positive subplate layer is in continuation with the underlying AChE-positive fibre system of the capsula interna and fibre bundles which penetrate the striatal anlage (Fig. 1).

In fetuses of 24–26 weeks of gestation the AChE-positive reaction appears in the middle third of the cortical plate (Fig. 4 asterisk). This intensive AChE staining appears to be

Fig 2 AChE staining in the subplate layer and marginal zone (MZ) in the auditory cortex of 22-week human fetus (arrows). In the cortical plate weak AChE staining (asterisk). Bar = 200 µm marks magnification for Figs. 1–7.

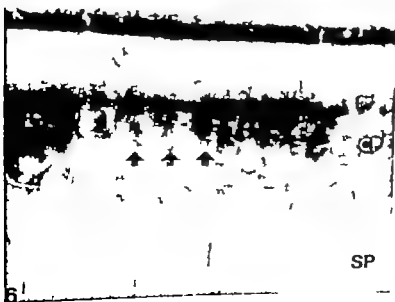
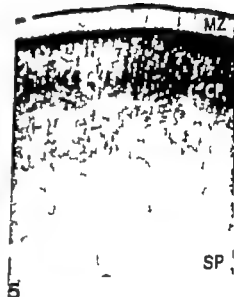
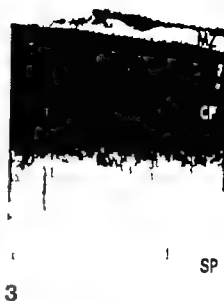
Fig 3 Nissl-stained section (adjacent) from specimen shown in Fig. 2.

Fig 4 Auditory cortex. Human fetus, 25 weeks. AChE staining. Note increased AChE staining in the cortical plate (asterisk).

Fig 5 Nissl-stained adjacent section.

Fig 6 Darkly stained AChE-positive alternating (arrow) zones ('columns') in the prospective layer IV of auditory cortex of the 28-week human fetus.

Fig 7 Nissl-stained (adjacent) section. Human fetus, 28 weeks.



critically uniform. There was a decrease in the density of AChE staining within the subplate layer (Fig. 4).

In oldest fetuses examined (28 weeks of gestation) a pattern of vertical darkly stained alternating zones (140–200  $\mu\text{m}$  wide) appeared within the AChE-positive middle third of the cortical plate (Fig. 5 arrows). These vertical positive zones (columns) are separated by narrower interspaces of lower staining density. AChE-positive columnar zones could be followed in serial sections in a rostrocaudal direction.

## DISCUSSION

The observation presented in this paper provides evidence that there is a characteristic distribution and evolution of AChE staining within cytoarchitectonic compartments of the developing human auditory cortex. The early bilaminar pattern of AChE staining (marginal zone and subplate layer) corresponds to the bilaminar distribution of synapses (Molliver, Kostović & Van der Loos 1973; Kostović, Knežević, Kostović, Krmpotić, Nemančić, Kelović & Vuković, 1978) observed in human fetal cortex. Thus AChE-positive fibres may be involved in early fetal synaptogenesis. The onset of AChE staining within the cortical plate at 24 weeks of gestation corresponds to the appearance of synapses in this layer (Molliver, Kostović & Van der Loos 1973; Kostović & Molliver 1974) and may indicate the establishment of thalamocortical circuitry. In accordance with this hypothesis is our finding of AChE-rich, vertically oriented alternating zones in prospective layer IV of the auditory cortex at 28 weeks of gestation. This columnar pattern of AChE staining which has been also observed by us in frontal cortex of human fetus (Kostović 1979) may correspond to the columnar distribution of thalamocortical fibres found in primate neocortex (Hubel & Wiesel 1969; Rakic 1976) or granule cells in primate auditory cortex (Smith & Moskowitz, 1979) while AChE-poor zones may correspond to the termination of cortico-cortical

fibres observed in the frontal cortex by Goldman & Nauta (1977). Vertically oriented AChE-positive bands may be also related to the columnar cortical organization identified by both physiological and neuroanatomical approaches (Mountcastle 1957; Hubel & Wiesel 1977; Hubel, Wiesel & Stryker 1978). Thus our observations (Kostović 1979; this study) may constitute the first evidence of columnar organization in the human cerebral cortex.

As suggested previously (Vaughan 1975; Krmpotić, Nemančić, Kostović, Nemančić & Kelović, 1979) the early establishment of the main organization of the auditory cortex may be of essential significance for both normal and abnormal development of the central auditory system.

At present we have no evidence that the AChE-positive reaction found in our material may reflect the cholinergic nature of the thalamic input to auditory cortex as was proposed for other sensory cortices (Kristit 1979). It is possible that the presence of AChE is related to the process of growth and differentiation and may be present in some developing telencephalic fibre systems (Kostović, Knežević, Kostović, Krmpotić, Nemančić & Kelović 1979; Kostović, Kelović, Kračun & Krmpotić, Nemančić 1979). Furthermore the AChE-positive reaction may be related to some non-cholinergic inputs as suggested by Emson & Lindvall (1979).

## RÉSUMÉ

Dans cette étude les méthodes de coloration avec acétylcholinestérase (AChE) étaient adoptées pour démontrer le développement de fibres afférentes du futur cortex auditif dans les fœtus humains de 8 à 28 semaines de gestation. La première AChE positive réaction apparaît chez les fœtus de 20–4 semaines dans le neuropile de la zone marginale et dans le subplate layer du cortex auditif. Par cette coloration caractéristique avec AChE le cortex auditif peut être délimité des autres régions corticales. À 4–28 semaines pendant la laminarisation latérale de la zone corticale AChE-positive réaction fut trouvée dans la partie profonde de la zone corticale. Dans les stades de 28 semaines une structure en colonnes verticales et sombres dans le tiers intermédiaire de la zone corticale fut observée. On peut en conclure que les fibres

AChE positives ont une distribution caractéristique dans les compartiments cytoarchitectoniques et couches synaptique du cortex auditif en développement. Les couches AChE positives coïncident avec la structure laminaire de la synaptogenèse. Cette réaction pendant le développement peut servir comme indicateur de l'innervation corticale afférente (thalamique) du cortex auditif. Cette réaction pourrait indiquer la nature spécifique du médiateur des fibres afférentes dans le cortex auditif.

## ZUSAMMENFASSUNG

In dieser Studie wurden die Acetylcholinesterasefärbungsmethoden (AChE) verwendet um die Entwicklung der prospektiven afferenten Fasern des auditiven Cortex in menschlichen Feten von 8-28 Schwangerschaftswochen zu demonstrieren. Erste AChE-positive Färbung wurde bei Feten in 20 - 4 Woche im Neopall der marginalen Zone und im „subplate layer“ des auditiven Cortex gefunden. Durch diese charakteristische AChE Färbung kann der auditive Cortex von den umgebenden corticalen Flächen unterschieden werden. In den 4 - 6 Wochen während der intensiven Lamination der Cortikalplatte wurde die AChE-positive Färbung in der tiefen Schicht der Cortikalplatte gefunden. In den älteren Stadien (28 Schwangerschaftswochen) wurde eine säulenförmige Struktur aus vertikalen sich dunkel färbenden Zonen im mittleren Drittel der Cortikalplatte gefunden. Wir können mit der Feststellung schließen daß die AChE Färbung in den cytoarchitektonischen Flächen und „synaptischen Schichten“ des sich entwickelnden auditiven Cortex charakteristisch verteilt ist. Die AChE-positive Schichten fallen mit dem laminiären Muster der Synptogenese zusammen so daß diese Reaktion während der Entwicklung als Parameter der corticalen afferenten (thalamischen) Innervation des auditiven Cortex dienen kann. Die AChE Reaktion könnte auf eine spezifische Transmitter der afferenten Fasern des auditiven Cortex hinweisen.

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# DIE BEDEUTUNG DER ZELLPROLIFERATION UND HISTOMORPHOLOGIE BEI DER GEPLANTEN BEHANDLUNG DER KEHLKOPFKARZINOME

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**Zusatz:** Die Differenzierungsmöglichkeit der einzelnen Phasen des Zellzyklus und die Kenntnisse über proliferationskapazität der Gewebe erwiesen sich als bedeutend zur besseren Erkennung des biologischen Verhaltens der Tumore. Neben den klinischen Eigenschaften (Lokalrezidiv, TNM Stadium) und des Ökotyps der Geschwulst sind auch die zellkinetischen Parameter sehr wichtig. Es wurde bei Kehlkopfkrebspatienten der Zusammenhang zwischen dem Malignitätsgrad und der zellkinetischen Daten untersucht. Aus der Bestimmung des Markierungsindex (L.I.) und der DNA Syntheserate und auch die übrigen populationskinetischen Angaben zu erfinden. Es konnte bei der autoradiographischen Untersuchung von 78 Kehlkopftumoren ein Zusammenhang zwischen der Größe des L.I. und dem biologischen Verhalten des Tumors gefunden werden. Es konnten bei den wenig differenzierten oder anaplastischen Karzinomen immer höhere L.I. feststellen. Zusammen mit den klinischen Angaben des Karzinoms steht der erhöhte L.I. auf die Möglichkeit der histogenen Metastasierung hin. Auf Grund der zellkinetischen Daten ergibt sich eine bessere differenzierte Therapie. Man kann auf der Wirksamkeit der präoperativen strahlens- und physikalischen Behandlung folgen.

## MATERIAL UND METHODE

Zu den zellkinetischen Untersuchungen wurde  $^3\text{H}$  Thymidin ( $^3\text{H}$ [TdR]) angewandt.

Mit in vivo und in vitro Incorporation der Radionucleosid bestimmten wir in 7 Fällen die Zeit der DNS-Synthesephase ( $T_s$ ) und die Werte des Markierungsindex (L.I.).

Vor der Biopsie spritzten wir direkt in den Tumor  $30 \mu\text{Ci } ^3\text{H}$ [TdR] bei den leicht zugänglichen supraglottischen Geschwülsten. (Die Lösung wurde mit Toluidin-blau gefärbt.) Nach 30 Minuten geschah die erste Exzision. Das Gewebestück wurde histologisch und autoradiographisch aufgearbeitet. Zwei Stunden später folgte die zweite Probeexzision aus dem markierten Gebiet, dann wurde noch für 30 Minuten in eine auf  $37^\circ\text{C}$  temperierte  $\alpha$  MEM (minimum essential medium) inkubiert. Die Lösung enthielt  $5 \mu\text{Ci/ml } ^3\text{H}$ [TdR] und wurde mit Sauerstoff durchgeströmt. Anschliessend erfolgte die Fixierung und Einbettung. Die Schnitte wurden entparaffiniert und mit Ilford G-5 Emulsion bedeckt. Nach wöchiger Expositionszeit wurden die Schnitte entwickelt, fixiert und mit Hämatoxylin-Eosin (H.E.) gefärbt.

Aus dem Unterschied der L.I. Werte der in vivo - in vitro markierten und nur in vivo markierten Präparaten ist es möglich die Zellströmung bzw. den Fluxus der DNS-synthetisierenden Zellen zu bestimmen. (An Hand von der Methode von Galand, Lala und Muggia.)

Auf der 1. Abbildung sind die vereinfachten Formeln zu sehen. Mit Hilfe dieser Gleichungen kann der L.I. Der Mitoseindex (M.I.) aus dem Zellfluxus während einer Stunde die

In den vergangenen 3 Jahrzehnten wurde wiederholt über die Bedeutung der Proliferationskinetik, normaler und maligner Gewebe berichtet.

Zugrunde liegt die Unterteilung des Zellzyklus nach Howard & Pelc in die intermitotische ( $G_0$  -  $S$  -  $G_2$ ) und mitotische (M) Phase und die Unterteilung der Zellpopulation in die proliferierende und nichtproliferierende Fraktionen - nach Mendelsohn (Growth fraction).

Proliferative Eigenschaften der Geschwülste erwiesen sich als bedeutend zur besseren Erkennung des biologischen Verhaltens maligner Tumoren, deshalb untersuchten wir zusammen mit den histomorphologischen Eigenschaften der Laryxstumoren - die Bedeutung der Zellkinetik.

- 1  $L I = \frac{NS}{N} \cdot 100$  <sup>3H</sup> MARKIERUNGSINDEX  
LABELLING INDEX
- 2  $M I = \frac{N_{M}}{N} \cdot 100$  MITOSEINDEX
- 3  $\Delta L I = \frac{L I (IN VIVO + IN VITRO) - L I (IN VIVO)}{t}$   
 $\Delta L I$  = ZELLFLUSS WÄHREND EINER STUNDE
- 4  $T_S = \frac{L I (IN VIVO)}{\Delta L I}$  DNS SYNTHESIZEIT
- 5  $T_C \approx T_S \times 3-4$  GENERATIONSZEIT
- 6  $PR = \frac{T_S}{T_C} \cdot L I$  PROLIFERATIONSRATE  
PR = CF GROWTH FRACTION
- 7  $DT POT = \frac{T_S}{L I}$  POTENTIELLE VERDOPPELUNGSZEIT  
 $\Delta C$  = GROWTH CONSTANT (STEEL)
- 8  $\phi = 1 - \frac{DT POT}{DT}$  ZELLVERLUST (CELL LOSS)

Abb 1 Formeln, die im Vortrag vorkommen

Ts Zeit bestimmt werden. Mit Bezugnahme auf die Publikationen von Fabricant, Friedel, Kissel, Klein, Schultze, Steel, Terz, Young kann die Tc 3-4 mal länger als die Ts angesehen werden.

An Hand der zellkinetischen Angaben sind die populationskinetischen Parameter, die

Grösse der proliferierenden Fraktionen (G.F. und die potentielle Verdoppelungszeit (T.I. pot.) zu bestimmen (Steel).

Mit Hilfe der Beobachtungen der Patienten, die nach der Probeexzision monatelang jederlei Behandlung zurückweisen ist es möglich, die Zunahme des Tumors zu beurteilen. Daraus kann die klinische Verdoppelungszeit (D.T.) ausgerechnet werden. In Abhängigkeit der klinischen und potentiellen Verdoppelungszeiten ist die Grösse des Zellverlustes (Cell loss) zu bestimmen (Steel).

Eine der wichtigsten zellkinetischen Angaben ist der L.I. (Fabrikant). Dieser Parameter wird aus dem Biopsiematerial mit <sup>3</sup>H[TdR] Markierung festgelegt (Helsa, Nitze, Rajewsky).

Mit diesem Verfahren kann man mit Sicherheit die in dem gegebenen Zeitpunkt DNS synthetisierenden Zellen markieren, wobei auch wichtig ist, dass es keine extra Belastung oder Gefahr für die Patienten bedeutet. Zwischen 1974-1978 wurde der L.I. bei 71 Kranken bestimmt.

Gleichzeitig wurde auch der Mitoseindex (M.I.) festgelegt. Das Geschwulstgewebe wurde sofort ohne Inkubation fixiert, histologisch aufgearbeitet und mit H.E. oder nach Feulgen gefärbt.

Tabelle I Nach in vivo-in vitro Incorporation von <sup>3</sup>H[Thymidin] bestimmte Markierung indices und DNS Synthesezeit bzw. geschätzte zellkinetische Parameter der Larynxkarzinome

|  | Differenzierungsgrad |          |      |                                 |            |                       |
|--|----------------------|----------|------|---------------------------------|------------|-----------------------|
|  | Hoch-differenziert   |          |      | Mittelmässig (moderately) diff. |            | Gering (poorly) diff. |
| Zahl der Fälle   | 3                    |          |      | 2                               |            |                       |
| <sup>3</sup> H[TdR] Markierungsindex in vivo L.I.                              | 5.0                  | 6.3      | 7.5  | 7.8                             | 9.6        | 10.1                  |
| <sup>3</sup> H[TdR] Markierungsindex in vivo + in vitro ( <sup>3</sup> H) L.I. | 5.7                  | 6.84     | 8.4  | 8.9                             | 10.8       | 11.36                 |
| Zellfluss/Stunde/ $\Delta L I$   | 0.35                 | 0.27     | 0.45 | 0.35                            | 0.6        | 0.63                  |
| DNS-Synthesezeit S-phase   | 7.9                  | 4.3%     | 6.6  | 7.9                             | 6.3%       | 6.2%                  |
| Generationszeit T.C  | 14.3                 | 23.3     | 16.6 | 14.2                            | 16.0       | 16.0                  |
| Proliferationsrate P.R. G.F.   |                      | 63       |      |                                 | 53         | 61                    |
| Pot. Verdoppelungszeit D.T. POT  |                      | 0.22     |      |                                 | 0.31       | 0.41                  |
| Akt. Verdoppelungszeit   |                      | 1.88 (1) |      |                                 | 1.74 (7.3) | 1.48 (6...)           |
| Zellverlust, $\phi$  |                      | ~180 Tag |      |                                 | ~150 Tag   | ~120 Tag              |
|  |                      | 93%      |      |                                 | 95%        | 95%                   |

Tabelle II  $^3\text{H}$  Thymidin Markierungsindex und die histopathologischen Angaben der Larynxkarzinome 1974-1978

|   | Differenzierungsgrad |                               |  |
|---|----------------------|-------------------------------|--|
|   | Hochdifferenziert    | Mittelmäßig (moderately) diff | Gering differenziert und undifferenziert |
| Cases                                   | 79                   | 11                            | 13                                       |
| $^3\text{H}$ Markierungsindex, L.I. (%) | (5-7,8) 6,5          | (7-10,5) 8,5                  | (9,8-15,1) 11,9                          |
| Markierungsindex, M.I. (%)              | (0,7-1,3) 1,06       | (0,9-1,1) 1,03                | (1,5-4,1) 2,9                            |
| Zellkern-Markierung (nuclear grading)   | NG 3                 | NG 3-NG                       | NG 1                                     |
| Necrosis                                | 0 +                  | +                             | +  |
| Invasion                                |                      | Gering (low)                  | Tief (extensive)                         |
| (in der Randzone)                       | Unregelmäßig         | (irregular)                   | (highly)                                 |

Es sei betont, dass der M.I. inkubierten Gewebes immer geringer als in vivo-Verhält-nissen ist, weil die im Gang befindlichen Mitosen während der Inkubation ablaufen aber neue Zellen aus der G<sub>2</sub>-Phase in die M-Phase nicht übertreten (Rajewsky, Oehlert).

### ERGEBNISSE

Auf Grund des histologischen Differenzierungsgrades sind die Kehlkopfpatienten in 3 Gruppen eingeteilt worden:

- in der I Gruppe: Kranken mit hochdifferenzierten,
- in der II Gruppe: Kranken mit mittelmäßig differenzierten
- in der III Gruppe: Kranken mit gering differenzierten Karzinomen.

Dem Reifegrad entsprechend wurden auch die Zell- und populationskinetischen Parameter gruppiert.

Die Tabelle I zeigt die mit in vivo und in vitro  $^3\text{H}$ [Tdr] Markierung bestimmten und geschätzten Werte.

Die Werte der Markierungsindices sind innerhalb einer Gruppe auch nicht gleich, trotzdem korrelieren sie mit dem Differenzierungsgrad des Tumorgewebes gut.

Die Wachstumsfraktion (GF) erweist sich als eine andere charakteristische Angabe. Mit Abnahme der Ausreifung der Tumorzellen steigt die Zahl der proliferierenden Zellen

des Gewebes. Es entspricht der von Stein abgefasster Grundregel mit der Abnahme der strukturellen Differenzierung nimmt die funktionelle Kapazität ab und es steigt die reproduktive proliferative Kapazität des Gewebes.

Der Zellverlust beträgt mehr als 90% stammt aus Zelltod, Migration und die Exfoliation spielt bei den Larynx Tumoren eine wesentliche Rolle.

Die nach in vitro  $^3\text{H}$ [Tdr] Incorporation bestimmten Angaben von 78 L.I. wurden nach dem strukturellen Differenzierungsgrad gruppiert. Die Ergebnisse sind in der II Tabelle dargestellt.

Die Werte der Markierungsindices sind mit dem histopathologischen Befunden in gutem Einklang und ändern sich zwischen 5-15%. Ein L.I. der unter 8% liegt ist für die gut differenzierten Geschwülsten charakteristisch. Der L.I. wurde nur dann über 10% gefunden wenn das Karzinom geringdifferenziert oder undifferenziert war.

Mit den anderen morphologischen Kennzeichen der Malignität des Tumors zeigte der L.I. eine gute Korrelation.

Die Zellkernatypie ist bei den hochdifferenzierten Geschwülsten nach Bloom und Black (NG3) geringer aber mit der Abnahme des strukturellen Reifegrades können sowohl in Form, als auch in der Größe und Färbung bedeutende Unterschiede gefunden werden (Pleomorphismus NG<sup>2</sup> NG1).

Die Ausdehnung der Nekrose bei nicht



$$1 \quad L I = \frac{N S}{N} 100 \quad \text{3\# MARKIERUNGSINDEX LABELING INDEX}$$

$$2 \quad M I = \frac{N M}{N} 100 \quad \text{MITOSEINDEX}$$

$$3 \quad \Delta L I = \frac{L I (IN VIVO + IN VITRO) - L I (IN VIVO)}{t}$$

$$\Delta L I = \text{ZELLFLUXUS WÄHREND EINER STUNDE}$$

$$4 \quad T_S = \frac{L I (IN VIVO)}{\Delta L I} \quad \text{DNS SYNTHESIZEIT}$$

$$5 \quad T_C = T_S \times 3-4 \quad \text{GENERATIONSZEIT}$$

$$6 \quad P R = \frac{T_C}{T_S} L I \quad \text{PROLIFERATIONSRATE PR CF GROWTH FRACTION}$$

$$7 \quad D T_{POT} = \frac{1}{L I} \quad \text{POTENTIELLE VERDOPPELUNGSZEIT}$$

$$L I = \text{GROWTH CONSTANT (STEEL)}$$

$$8 \quad \Phi = 1 - \frac{D T_{POT}}{D T} \quad \text{ZELLVERLUST (CELL LOSS)}$$

Abb 1 Formeln die im Vortrag vorkommen.

Ts Zeit bestimmt werden Mit Bezugnahme auf die Publikationen von Fabricant frindel Kissel Klein Schultze Steel Terz Young kann die Tc 3-4-mal länger als die Ts angesehen werden

An Hand der zellkinetischen Angaben sind die populationskinetischen Parameter die

Grösse der proliferierenden Fraktionen (G F) und die potentielle Verdoppelungszeit (D T pot) zu bestimmen (Steel)

Mit Hilfe der Beobachtungen der Patienten, die nach der Probeexzision monatelang jeder bei Behandlung zurückweisen ist es möglich die Zunahme des Tumors zu beurteilen. Daraus kann die klinische Verdoppelungszeit (D T) ausgerechnet werden In Abhängigkeit der klinischen und potentiellen Verdoppelungszeiten ist die Grösse des Zellverlustes (Cell loss) zu bestimmen (Steel)

Eine der wichtigsten zellkinetischen Angaben ist der L.I (Fabrikant) Dieser Parameter wird aus dem Biopsiematerial mit in vitro  $^3\text{H}[\text{TdR}]$  Markierung festgelegt (Helap. Nitzte Rajewsky)

Mit diesem Verfahren kann man mit Sicherheit die in dem gegebenen Zeitpunkt DNS-synthetisierenden Zellen markieren wobei auch wichtig ist dass es keine extra Belastung oder Gefahr für die Patienten bedeutet. Zwischen 1974-1978 wurde der L.I bei 78 kranken bestimmt

Gleichzeitig wurde auch der Mitoseindex (M I) festgelegt Das Geschwulstgewebe wurde sofort ohne Inkubation fixiert histologisch aufgearbeitet und mit H E oder nach Feulgen gefärbt

Tabelle I Nach in vivo-in vitro Incorporation von  $^3\text{H}[\text{Thymidin}]$  bestimmte Markierungsindices und DNS Synthesizeit bzw geschätzte ellkinetische Parameter der Larynxkarzinome

|   | Differenzierungsgrad |      |      |                                |      |       |                       |  |
|---|----------------------|------|------|--------------------------------|------|-------|-----------------------|--|
|   | Hoch-differenziert   |      |      | Mittelmässig (moderately) diff |      |       | Gering. (poorly) diff |  |
| Zahl der Fälle  | 3                    |      |      | 2                              |      |       |                       |  |
| $^3\text{H}[\text{TdR}]$ Markierungsindex in vivo L.I                         | 5.0                  | 6.3  | 7.5  | 7.8                            | 9.6  | 10.1  | 13.2                  |  |
| $^3\text{H}[\text{TdR}]$ Markierungsindex in vivo + in vitro ( $^{3\#}$ ) L.I | 5.7                  | 6.84 | 8.4  | 8.9                            | 10.8 | 11.36 | 14.6                  |  |
| Zellfluxus/Stunde/ $\Delta L I$   | 0.35                 | 0.27 | 0.45 | 0.35                           | 0.6  | 0.63  | 0.71                  |  |
| DNS-Synthesizeit S-phase  | 7%                   | 4.3% | 6%   | 7%                             | 6.3% | 6.4%  | 5.4%                  |  |
| Generationszeit TC  | 14.3                 | 23.3 | 16.6 | 14.2                           | 16.0 | 16.0  | 18.6                  |  |
| Proliferationsrate P.R. G F   | 63                   |      |      | 33                             |      |       | 61                    |  |
| Pot. Verdoppelungszeit DT POT   | 0.22                 |      |      | 0.31                           |      |       | 0.41                  |  |
| Akt Verdoppelungszeit   | 288 (12)             |      |      | 174 (7.3)                      |      |       | 143 (6.4)             |  |
| Zellverlust, $\Phi$   | ~180 Tag             |      |      | ~150 Tag                       |      |       | ~120 Tag              |  |
|   | 93%                  |      |      | 95%                            |      |       | 95%                   |  |

Tabelle IV. TNM System (UICC) des Kehlkopfs erga.nzt mit dem Differenzierungsgrad und H Markierungsindex der Geschwulste

D<sub>1</sub> Hoch-mittelmässig differenzierte Geschwulste L.I. <9  
D<sub>2</sub> Geringdifferenzierte und anaplastische Geschwulste L.I. >9

| Stadium    | T              | N <sub>0</sub> | N <sub>1a</sub> | V N <sub>2a</sub> , M <sub>0</sub>                 | D <sub>1</sub><br>D <sub>2</sub> |
|------------|----------------|----------------|-----------------|--|----------------------------------|
| I Stadium  | T <sub>0</sub> | N <sub>0</sub> | N <sub>1a</sub> | V N <sub>2a</sub> , M <sub>0</sub>                 | D <sub>1</sub><br>D <sub>2</sub> |
| II Stadium | T <sub>0</sub> | N <sub>0</sub> | N <sub>1a</sub> | V N <sub>2a</sub> , M <sub>0</sub>                 | D <sub>1</sub><br>D <sub>2</sub> |
|            | T              | N <sub>0</sub> | N <sub>1a</sub> | V N <sub>2a</sub> , M <sub>0</sub>                 |                                  |
|            | T <sub>0</sub> | T              | N <sub>1a</sub> | V N <sub>2a</sub> , M <sub>0</sub>                 |                                  |
| IV Stadium | T              | T <sub>0</sub> | T               | V T <sub>0</sub> , N <sub>2</sub> , M <sub>0</sub> | D <sub>1</sub><br>D <sub>2</sub> |
|            | T              | T              | T               | V T M  |                                  |
|            | N <sub>0</sub> | N              | N <sub>2</sub>  | V N  |                                  |

Ergebnissen von 78 Kehlkopftumoren konnte ein Zusammenhang zwischen der Grösse des L.I. und dem biologischen Verhalten des Tumors gefunden werden. Wenn der L.I. eines Karzinoms unter 8% ist ist sein Malignitätsgrad niedrig über 10% ist der Malignitätsgrad des Tumors hoch. Wir fanden bei den wenig differenzierten oder anaplastischen Karzinomen immer hohe Markierungsindizes und proliferierende Fraktionen.

Zusammen mit den klinischen Angaben des Tumors weist der Onkotyp und die mitotische Aktivität auf die Häufigkeit der Metastasen hin (Bauer).

Je mehrere Angaben in einem konkreten Fall bekannt sind desto sicherer kann man die prognostischen und therapeutischen Folgerungen ziehen. Alderson fand bei Mammakarzinomen Ferrillo bei Larynx und Hypopharynxkarzinomen die multifaktorielle Analyse als ergebnisvoll.

Die 2. Abbildung zeigt unseren Standpunkt bei der Beurteilung der Larynxkarzinome.

Mit Hilfe der histologischen und zellkinetischen Angaben ergänzten wir das von UICC eingeführte TNM System mit dem Differenzierungsgrad und L.I. des Larynxkarzinoms.

Mit D wurden die hoch oder mittelmässig differenzierten Karzinome mit einem L.I. unter 9% bezeichnet.

Mit D<sub>2</sub> wurden die gering oder undifferenzierten Karzinome mit einem L.I. über 9% bezeichnet.

So ergab sich die Möglichkeit dass die in dasselbe Stadium gehörenden Kranken doch in 2 Gruppen separiert sein konnten.

59 von den erwähnten Patienten haben schon mindestens 3-jährige Behandlungsergebnisse. Ein signifikanter Unterschied konnte zwischen der D<sub>1</sub> and D<sub>2</sub> Gruppe festgestellt werden.

Tabelle V. 3-Jährige Überlebensrate auf Grund der TNM Stadium Differenzierungsgrad und H Markierungsindex der Larynxkarzinome (D und D<sub>2</sub>)

| I Stadium           | II Stadium               |                | III Stadium               |                | IV Stadium     |                |
|---------------------|--------------------------|----------------|---------------------------|----------------|----------------|----------------|
|                     | D <sub>1</sub>           | D <sub>2</sub> | D                         | D <sub>2</sub> | D <sub>1</sub> | D <sub>2</sub> |
| Kern Astoradiagramm | 6/7<br>100%              | 2/6<br>67%     | 2/22<br>91%               | 4/13<br>60%    | 2/6<br>67%     | 2/2<br>0%      |
| Insgesamt           | D <sub>1</sub> 4/33, 89% |                | D <sub>2</sub> 10/74, 58% |                |                |                |

Table III: Zusammenhang zwischen histologischer Klassifikation +  $^3\text{H}[\text{TdR}]$  Markierungsindex und der klinischen Angaben der Larynxkarzinome

|  | Malignitätsgrad |                                    |                    |
|--|-----------------|------------------------------------|--------------------|
|  | I Gering (low)  | II Übergangsstadium (intermediate) | III-IV Groß (high) |
| Casus                                  | 29              | 31                                 | 11                 |
| H Markierungsindex L.I                 | 6,5             | 8,5                                | 11,9               |
| Wachstum                               | 65% Exophyt     | Exophyt-Endophyt                   | Endophyt           |
| Tastbare Lymphknoten vor der Operation | 9/29 17%        | 15/31 48%                          | 13/18 72%          |
| Überlebensrate (3-Jährig)              | 17/21 90%       | 3/25 88%                           | 9/13 31%           |

behandelten Karzinomen ist mit der Proliferationsgeschwindigkeit im Verhältnis (je schneller desto grösser)

Das Bild des Marginalgebietes zwischen den normalen und malignen Gewebe zeigt sich auch als charakteristisch. Die Tiefe der Invasion und besonders des Grad der Rundzellinfiltration sind von grosser Bedeutung. Es wurde der Zusammenhang zwischen dem L.I. und den klinischen Angaben untersucht.

Auffallend und wichtig ist die Beziehung zwischen dem L.I. und dem Vorkommen der vor der Operation vergrösserten Lymphknoten. Mit niedrigen L.I. fanden wir selten Lymphknoten dagegen wenn der L.I. über 10% war, konnten wir sehr oft Lymphknoten

tasten. Die Metastasengefahr ist bei den schnellproliferierenden Tumoren viel häufiger. Dies stimmt mit den Beobachtungen von Arthur McGavran und Sessions zusammen, die einen signifikanten Zusammenhang zwischen dem Vorkommen der Lymphknotenmetastasen und dem strukturellen Reststadium der Tumoren fanden.

## DISKUSSION UND THERAPEUTISCHE FOLGERUNGEN

Neben den klinischen Eigenschaften des Kehlkopfkarzinoms (Sitz, Grösse, Metastasen) sind die histologischen, zellkinetischen Parameter und die Immunkapazität des Organismus von grosser Bedeutung. Obwohl das TNM System sich als nützlich beweist, ist eine an breiter Basis ruhende Klassifikation notwendig, um die Proliferationsaktivität und die Aggressivität des Tumors zu beurteilen und um einen besseren Therapieplan finden zu können.

Es wurde bei Kehlkopfkarzinomen ein enger Zusammenhang zwischen dem Malignitätsgrad (struktureller Differenzierung, Pleomorphismus der Zellen, Nekrosis, Invasion und Mitosenzahl) und dem zellkinetischen Parameter gefunden. Aus der Bestimmung des L.I. und des TS sind auch die populationskinetischen Angaben zu erfinden (potentielle Verdoppelungszeit, proliferierende Fraktion, Zellverlust).

Bei den autoradiographischen Untersu-

### A. KLINISCHE EIGENSCHAFTEN

1. LOKALISATION
2. TNM KLASSE
3. MAKROSKOPISCHE ASPEKTE

### B. HISTOMORPHOLOGISCHE EIGENSCHAFTEN

1. ORTOTYP
2. MALIGNITÄTSGRAD (I-III)

### C. IMMUNOMORPHOLOGISCHE EIGENSCHAFTEN

1. MARGINALE UND INTRATUMORALE RUNDZELL-INFILTRATION
2. REAKTIONSTYP (ZELLULÄR, HUMORAL) DER VERGRÖSSERTEN LYMPHKNOTEN

### D. ZELLMINETISCHE PARAMETER

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Bei den D<sub>2</sub> Tumoren ergab sich die 3-jährige Überlebensrate um 31% niedriger als bei den D<sub>1</sub> Tumoren. Daraus muss eine wichtige Konsequenz gezogen werden: die Kranken, die einen geringdifferenzierten und schnell proliferierenden Tumor haben, befinden sich in grösserer Gefahr und brauchen eine viel seitigere und gründlich geplante Therapie. In diesen Fällen sind die rationellen Kombinationsmöglichkeiten der chirurgischen Strahlentherapie- und zytostatischen Behandlung besonders wichtig.

Es ist schon seit langem bekannt, dass eine Korrelation zwischen der Proliferationsaktivität und der Radiosensibilität besteht (Bloom, Coutard, Fletcher, Rubin). Je schneller das Wachstum ist, desto grösser ist die Wirkung der ionisierenden Strahlen. Die schneller proliferierenden und geringdifferenzierten D<sub>2</sub> Larynxkarzinome sind mit grösserer Wahrscheinlichkeit radiosensibler, darum bekommt die Bestrahlung bei diesen Kranken eine wichtige Rolle.

In Abhängigkeit von den Ergebnissen der multifaktoriellen Analyse wurde unser Therapieplan zusammengestellt wie folgt:

#### I Bei der Klasse T<sub>1</sub>

- 1 In der Gruppe D<sub>1</sub> Operation bevorzugt
- 2 In der Gruppe D<sub>2</sub> Strahlentherapie

#### II Bei der Klasse T<sub>2</sub>

- 1 In der Gruppe D<sub>1</sub> Operation bevorzugt
- 2 In der Gruppe D<sub>2</sub> Strahlenbehandlung bis 4000 rad, dann eine Woche Pause.

Je nach der Tumorrückbildung wird entweder die Irradiation bis zu einer Gesamtdosis von 6000 rad fortgesetzt oder operiert.

#### III Bei der Klasse T<sub>3</sub>

- 1 In der Gruppe D<sub>1</sub> chirurgischer Eingriff ohne Bestrahlung
- 2 In der Gruppe D<sub>2</sub> kombinierte Therapie

Nach 3000 rad folgt innerhalb einer Woche die chirurgische Massnahme. Nach der Heilung wird die Radiotherapie komplettiert. Bei ausgedehnten Tumoren halten wir die Totalexstirpation für die Methode der Wahl.

#### IV Bei der Klasse T<sub>4</sub>

Immer kombinierte Behandlung. Nach 6000 rad Gesamtdosis folgt eine 4-wöchige Pause, dann der chirurgische Eingriff.

Besonders in dieser Gruppe können die Strahlensensibilitätsmodifizierenden Faktoren (Zytostatika, Metronidasol-Derivate, usw.) zur Anwendung gebracht werden (Chapman, Yuhas).

### SUMMARY

The separation of phases in cell division and the recognition gained in tissue population kinetics have contributed to enhance our knowledge of the biological behaviour of the tumours. Besides the determination of clinical characteristics (localization, TNM state) and the oncotype of a given tumour, findings obtained by cell-kinetic parameters seem to be of great importance. Relations between the degree of malignancy and the data obtained by autoradiography were studied in patients suffering from laryngeal cancer. On the basis of the simple clinical determination of L<sub>1</sub> and T<sub>2</sub>, estimation of further cell-kinetic parameters in a given tumour has become possible and the number of cells in the same cycle, the growth fraction and the potential doubling time can be deduced. After the radiographic investigation of 78 cases of laryngeal tumour, a relation was found between the extent of the labelling index and the biological behaviour of the tumour.

### RÉSUMÉ

La séparation des phases de division cellulaire et la reconnaissance de la cinétique de la population des tissus ont contribué considérablement à ce que nous connaissions mieux l'attitude biologique des tumeurs. À côté des caractéristiques biologiques et cliniques (la localisation, le stade en TNM) et la définition du type oncotopique, il est très important de connaître les paramètres de la cinétique cellulaire. Nous avons examiné le degré de la malignité chez des malades au cancer du larynx et la relation de cela aux informations reçues par la méthode autoradiographique. La technique de la définition de L<sub>1</sub> et T<sub>2</sub> est facile et donne la possibilité pour calculer des paramètres de la cinétique cellulaire d'un tumeur donné. À la base des données obtenues par la cinétique cellulaire nous avons la possibilité d'un traitement plus différencié dans des cas du tumeur du larynx et nous pouvons y tirer des conclusions concernant l'efficacité d'un traitement cytostatique et d'une irradiation préopérative.

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## STREPTOMYCIN INDUCED DEFECTS OF THE OTOCONIAL MEMBRANE

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**Abstract.** Damage to the neuro-epithelium caused by the vestibulotoxic drugs has been well described in previous reports, however little is known about the effect of such drugs on the otoconia. In this investigation, streptomycin sulfate is found to create a circumscribed defect in the cytoplasmic layer of the otoconial membrane in the utricle of the guinea pig. The defect was secondary to a sharply outlined lesion of the neuroepithelium which corresponded in size and location to the otoconial defect. Many of the otoconia along the margin of the defect showed signs of degeneration and appeared similar to those observed in the saccule of man during aging. In advanced stages of decay the otoconia were reduced to hollow shells consisting of longitudinal interconnecting strands. All the epithelial lesions occurred with remarkable consistency in the same region of the macula striola at the posterior end of the striola.

The aminoglycosides streptomycin, gentamicin and tobramycin are the most important vestibulotoxic drugs. Their major site of action is the neuro-epithelium and the damage they cause has been well documented (Hawkins, 1976). This report deals with injury to the utricular otoconia caused by streptomycin.

After intratympanic application of streptomycin, the sensory epithelium of the macula utricularis shows less damage than the ampullary crista and the macula sacculi still less (Lindeman, 1969). It is of interest to note that the Type I hair cells located within the striola (Werner, 1940) are more vulnerable than the Type II cells. Streptomycin is also known to affect the so-called dark cells in the membranous wall of the utricle (Hawkins & Preston, 1973).

Reports of otoconial abnormalities have been few. We have observed what probably represented abnormal saccular otoconia in

cats after gentamicin (Hawkins et al., 1969) and neomycin (Johnsson & Hawkins, 1977) treatment. Neomycin appeared to cause loss of otoconia on the posterior tip of the saccule while gentamicin created more generalized loss of saccular otoconia with some giant otoconia remaining. Because the specimens had been stored for several months in 70% alcohol the pH of which was not measured, the validity of these observations is open to question. Lim (1973) has observed abnormal otoconia in the guinea pig which he believes were altered due to ethacrynic acid treatment. Recently loss of both saccular and utricular otoconia has been observed in guinea pigs after administration of streptomycin (Harada & Sugimoto, 1977). As has been mentioned in our preceding report, experimentally induced otoconial abnormalities in guinea pigs have to be viewed in light of the fact that defective otoconial membranes do occur in untreated control animals (Johnsson et al., 1979).

This report deals with an otoconial defect which with certainty can be related to degenerative changes in the neuro-epithelium. The observation was made during dissection of the vestibular system in guinea pigs intended for use in a study of the effect of streptomycin on the incorporation of  $^{45}\text{Ca}$  in the otoconial membranes.

## METHODS

The technique of microdissection which was employed in the previous study (Johnsson et al., 1979) was also used in this investigation.



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**Abstract.** Damage to the neuro-epithelium caused by the otoblastic drugs has been well described in previous reports, however little is known about the effect of such drugs on the otoconia. In this investigation streptomycin sulfate was found to create a circumscribed defect in the crystalline layer of the otoconial membrane in the utricle of the guinea pig. The defect was secondary to sharply outlined lesions of the neuroepithelium which corresponded in size and location to the otoconial defect. Many of the otoconia along the margins of the defect showed signs of degeneration and appeared similar to those observed in the saccule of man during aging. In advanced stages of decay the otoconia were reduced to hollow shells consisting of longitudinal interconnecting strands. All the epithelial lesions occurred with remarkable consistency in the same region of the macula utriculi in the posterior end of the striola.

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After intratympanic application of streptomycin, the sensory epithelium of the macula utriculi shows less damage than the ampullary crista and the macula sacculi still less (Lundman 1969). It is of interest to note that the Type I hair cells located within the striola (Werner 1940) are more vulnerable than the Type II cells. Streptomycin is also known to affect the so-called dark cells in the membranous wall of the utricle (Hawkins & Preston, 1973).

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## METHODS

The technique of microdissection which was employed in the previous study (Johnsson et al 1979) was also used in this investigation.



and has been described in detail elsewhere (Hawkins & Johansson 1975). The original purpose was to investigate the uptake of  $^{45}\text{Ca}$  in the otoconial membranes; an extensive morphological study was not done. The specimens were fixed for approximately 24 hours in Zetterqvist's 1% osmium tetroxide solution and partially dehydrated in ascending concentrations of alcohol. The cochlea and the ampullary cristae were not studied. Most of the maculae were examined only in the dissection microscope. Surface preparations of the neuro-epithelia were prepared from a few maculae and viewed in phase-contrast illumination. The neuro-epithelia and the otoconial membranes of the utricular maculae from 4 guinea pigs were examined by scanning electron microscopy. The otoconial membranes were air dried and the maculae were  $\text{CO}_2$  critical point dried with amyl acetate as intermediate fluid.

## MATERIAL

Twenty-five albino guinea pigs were treated with subcutaneous daily doses of streptomycin sulfate powder (Pfizer) dissolved in saline 200 mg/kg for 19 to 20 days. The animals were killed at various intervals after the last drug dose. Marked ataxia was seen in 8 of 25 streptomycin-dosed animals. There was no correlation between the presence of ataxia and degree of depression of the pinna reflex. A subsequent series of 18 similarly treated guinea pigs was examined. The findings in this second group of animals were in all respects similar to those in the first group and will not be described in detail. Untreated guinea pigs used in other studies (Mechigian et al. 1979) served as control animals.

## RESULTS

### *Otoconial defects*

A striking circumscribed loss of otoconia was observed in the posterior half of the macula utriculi in several animals. No similar changes were found on the macula sacculi.

Because the guinea pigs were killed at various intervals after the last streptomycin dose it was possible to observe what appeared to be different stages and growth of the lesion after termination of the drug administration. With a few exceptions the size of the lesion was directly related to the time interval between the last injection and the day of sacrifice. The largest lesions were found in guinea pigs killed 20 days after the last dose. Lesions were present in all groups except in the animals killed 2 days after the injection; their neuro-epithelial and otoconial membranes all appeared normal under the dissection microscope.

The large defects were characterized by complete loss of otoconia in the middle of the lesion which left the gelatinous part of the membrane uncovered and translucent (Johnson & Hawkins 1967). This exposed the darkly stained macular tissue under the defect (Fig. 1). The gelatinous layer of the otoconial membrane appeared normal. The defects had an irregular shape and many otoconia at the margins showed varying degrees of staining, probably due to demineralization.

Examination of the margins by scanning electron microscopy revealed that some of the otoconia were in the process of degeneration. These crystals appeared remarkably similar to the degenerated otoconia observed in the human saccule in association with aging (Ros et al. 1976). Their surfaces were uneven with pitting and small grooves running longitudinally. In advanced stages of decay the otoconia were hollowed out leaving a shell of longitudinal interconnected strands. The end faces of the otoconia were less involved. In advanced cases the otoconia were split into two or three fragments (Fig. 2).

In guinea pigs killed 6 days after the last dose the changes in the otoconial membrane and the neuro-epithelium were even more circumscribed than in the other groups of animals and limited to a small round area. The otoconial membrane at this point formed a dome over the epithelial defect creating a small cavity between the defect and the underside of



Fig. 1. Utricle anaculae from two different animals. *T* the left the otoconial membrane has been dissected away to display the large epithelial defects. Note the close correlation in location and size between the epithelial lesions and defects in the otoconial membranes (shown

to the right). *A, B* large defects occurring 20 days after termination of streptomycin treatment (left ear). *C, D* small lesion (arrows) seen close to the posterior tip of the anacula 6 days after treatment (right ear). OsO

the membrane. There was no substantial loss of otoconia. Only a few minute holes in the otoconial layer surrounded by darkly-stained otoconia were seen at the top of the dome

#### Epithelial defects

In every specimen in which otoconial changes were present we found an epithelial defect, which corresponded in location and size to the defect in the otoconial layer. Often the epithe



Fig. 2 Scanning electron micrograph of otoconia located along the margin of a large defect seen 20 days after treatment. Different stages of otoconial degeneration are seen. Note the hollowed out otoconia with a shell of longi-

tudinal strands holding together the more solid-appearing ends of the crystals. Note also pitting and longitudinal fissures in earlier stages of degeneration (arrows).

lial defect was slightly larger than the involved area on the otoconial membrane. In a few specimens there were two small separate epithelial defects (Fig. 3). In those instances there were also two separate lesions on the otoconial membrane. The epithelial defects appeared as if they had been punched out. The larger lesions had thickened margins and were surrounded by several small satellite lesions. The tissue exposed by the defects was not covered by an epithelium. In some cases the neuro-epithelium in the anterior part of the macula was loosened from the underlying tissue and formed a wide bulge. These changes did not appear to result from artifacts and it is noteworthy that the otoconial layer above this particular region of damaged epithelium appeared completely intact under the dissection microscope.

All epithelial defects were located with remarkable consistency in the same area of the posterior half of the macula on the stria.

This was especially clearly demonstrated in specimens with smaller lesions. The characteristic location is shown in Fig. 1. In the samples studied the sensory cell loss was in general most severe in the stria.

#### *Animals with defective otoconial membranes*

In a previous study we found a high incidence of abnormal otoconial membranes in the utricle and saccule of untreated seemingly normal animals (Johnsson et al. 1979). Abnormal specimens showing a marked reduction of otoconia over the stria could easily be identified in both the treated and untreated guinea pigs used in the present investigation. In the animals with abnormal otoconial membranes streptomycin treatment produced epithelial defects identical with those described above. When the epithelial lesion was larger than the pre-existing otoconial defect loss of otoconia could be observed which

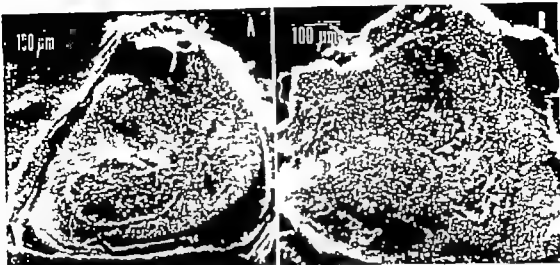


Fig 3 Scanning electron micrographs of the right utricular maculae from 2 animals killed 30 days after treatment. The tufts of sensory cells are displayed. The posterior tip of the macula is in the lower left and right corners

respectively. A. Single large lesion. The margin of the defect is thickened (critical arrow). B. Small defect (horizontal arrow). Not the sensory cell loss along the striola.

clearly accentuated the original defect in the otoconial membrane

### DISCUSSION

This study has demonstrated a more or less precise local correlation between a defect in the crystalline layer of the otoconial membrane and a streptomycin-induced lesion in the neuro-epithelium of the macula utriculi in guinea pigs. One can only speculate about the process which caused the loss of otoconia. It is highly improbable that purely mechanical factors were involved. We believe it is more likely that the epithelial damage interfered in some way with the calcium metabolism of the otoconia. Recent studies (Preston et al 1975; Mechuglan et al 1979) have provided evidence for active turnover of calcium in the otoconial membranes. Thus the streptomycin treatment may have disturbed biochemical mechanisms responsible for calcium transport or uptake. Also during degeneration of the neuro-epithelium, lysis of cells probably occurred leading to the release of lysosomal products and enzymes which may have low

ered the pH sufficiently to cause dissolution of the otoconia. Such products of cellular degeneration might in addition alter the organic matrix of the otoconia as well as the gelatinous portion of the otoconial membrane.

Our findings show little similarity to the otoconial changes recently attributed to streptomycin treatment by Harada & Sugimoto (1977). The degenerating otoconia in our specimens did appear similar to those found in the human saccule during aging (Ross et al 1976) which suggests that a common process might be involved in production of the two seemingly different forms of otoconial degeneration.

This study does not show whether loss of sensory cells and supporting elements alone unaccompanied by focal defects of the maculae can alter the otoconia. It seems likely that this will occur with time (Mechuglan et al 1979).

The marked loss of Type I sensory cells in the utricle was to be expected (Lindeman 1969) but the circumscribed lesion occurring consistently at the posterior end of the striola was not anticipated. This finding suggests the presence in the utricle of a *locus minoris resis*

tentiae for streptomycin much like that in the lower basal turn of the cochlea for most ototoxic drugs. Both the epithelial and the otoconial defects are unusual and could serve as useful tools in future morphological and functional studies on the macular organs.

## ACKNOWLEDGEMENTS

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## ZUSAMMENFASSUNG

Vestibulotoxische Neuroepithelschäden nach Aminoglykosidbehandlung sind von früheren Autoren beschrieben worden, doch weiß man wenig über die direkte Wirkung dieser Medikamente auf die Otolithen. In dieser Untersuchung wurde festgestellt, daß Streptomycin eine begrenzte Schädigung der Kristallschicht der Otolithenmembran der Macula utriculi verursacht. Diese Schädigung stimmte der Größe und Lage nach mit einem scharfdefinierten Defekt des Neuroepithels überein. Viele Otokonien am Rande des Defektes zeigten eine Art Degeneration wie sie bei der Macula sacculi des alternierenden Menschen beschrieben worden ist. Im fortgeschrittenen Degenerationsstadium waren die Otokonien ausgehöhlt, und bestanden aus ringförmigen miteinander verbundenen Strängen. Alle Defekte befanden sich mit bemerkenswerter Regelmäßigkeit an der gleichen Stelle der Macula utriculi d. h. am hinteren Ende der Striola.

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# THE NATURE OF THE OTOTOXIC ACTIONS OF ETHACRYNIC ACID UPON THE MAMMALIAN ENDOLYMPH SYSTEM

## I Functional Aspects

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**Abstract.** The endolymphatic changes produced by an intravenous injection of 60 mg kg<sup>-1</sup> ethacrynic acid were followed for up to 120 min using conventional and sensitive (Na<sup>+</sup>, K<sup>+</sup> and pH) microelectrodes in the rat. They were found to be caused by three distinct effects upon the endolymph system. Initially the drug completely abolished the stria potential-producing and cation-transporting processes. Recovery began quickly and was rapid at first. Then its rate declined considerably probably due to damage to stria energy production of delayed onset and prolonged duration. Coincident with these actions upon stria transport, there was decrease in the overall cation permeability of the endolymph system. This followed different time course and affected K<sup>+</sup> much more than Na<sup>+</sup>. The findings also provided further information about the mechanisms responsible for the normal endolymphatic composition. Qualitatively similar results were obtained in a subsidiary study on guinea pigs.

Reports of both transient and permanent deafness due to the use of ethacrynic acid began to appear in 1965 shortly after it was introduced into clinical practice. Since then its effects upon the inner ear have been studied extensively. As a result, it has been established that the stria vascularis is a major site of action of the drug and that significant changes occur in the cochlear endolymph especially in the endocochlear potential. However almost all of the investigations have been phenomenological in nature and comparatively little is known about the way ethacrynic acid produces the alterations which have been described.

One of its most important effects is a marked decrease in energy utilisation by the

stria vascularis which suggests that substantial inhibition of the active transport systems has probably taken place (Thalmann et al 1973 Kusakari et al 1978). There is also some diminution in energy production but this is relatively slight at the time of the maximal inhibitory action although the situation later has not been examined. The only stria ion-transporting enzyme so far identified a Na<sup>+</sup> K<sup>+</sup>-activated ATPase is relatively insensitive to ethacrynic acid and not much affected in vitro by the stria concentrations which inhibit the production of the endocochlear potential in vivo (Kuijpers & Willems 1976 Paloheimo & Thalmann 1977 Kusakari et al 1978). Direct inhibition of the Na<sup>+</sup> K<sup>+</sup>-activated ATPase is consequently unlikely to be a major cause of the drug-induced derangements. By contrast the dose-response curves of the inhibition of stria adenylate cyclase in vitro and the decrease in the endocochlear potential in vivo are approximately the same (Ahlström et al 1975 Paloheimo & Thalmann 1977). The presumed inhibition of the active transport processes may well be secondary therefore to depression of adenylate cyclase activity and an indirect effect upon the ATPase cannot be excluded. In addition there appear to be coincidental alterations in the permeability of the endolymphatic membranes but the evidence has not been sufficient for their precise nature to be determined (Bosher et al 1973 Melichar & Syka 1978).

The aim of the present investigation was to provide further information about the pathogenesis of ethacrynic acid ototoxicity by continuously following the endolymphatic changes produced in the cochlea by a single intravenous injection of the drug using conventional and ion sensitive microelectrodes. Sellick & Johnstone (1974) and Melichar & Syka (1978) have undertaken limited studies of this type upon respectively the endolymphatic  $\text{Na}^+$  or  $\text{K}^+$  concentration alone. In the absence of simultaneous measurements the interpretation of their findings has been greatly hampered so that the increase in our understanding of the underlying mechanisms has been disappointing. A deliberate effort was made accordingly to obtain such measurements in the experiments to be described. The results provided quantitative measurements of the three effects of ethacrynic acid which were found namely inhibition of the stria transport enzymes, decrease in membrane permeability and late depression of oxidative metabolism.

## METHODS

### *Animal preparation*

Caesarean-originated barrier sustained strains (Charles River) of Sprague Dawley albino rats (150–200 g) and Hartley albino guinea pigs (200–300 g) were used to ensure that the animals ears had not been subjected to the effects of either infection or antibiotics. Details of the anaesthesia and animal preparation will be found in the account of a previous investigation (Boshier 1979).

In brief two electrodes were routinely inserted into the cochlear duct through a small opening made over the stria vascularis of the left middle turn in the rat and left basal turn in the guinea pig. Surgical anaesthesia was maintained either with sodium pentobarbitone (rat) or with neuroleptanaesthesia (guinea pig) muscle relaxants were not used. It was necessary to keep the animals temperature constant (to  $\pm 0.4^\circ\text{C}$ ) by means of a ther-

mistor-controlled heating blanket because the ion-sensitive microelectrode readings were temperature sensitive.  $35^\circ\text{C}$  was chosen for the rat since higher temperatures caused hypernoea and  $37^\circ\text{C}$  for the guinea pig. Ethacrynic acid was injected as sodium ethacrylate solution (Edectrin Injection Merck, Sharp & Dohme) into the previously-exposed right femoral vein the injection time being 60 sec. Terminal anoxia was produced after varying intervals by the injection of 2 ml air through an indwelling catheter in the left femoral vein without disturbing the electrical screening around the preparation.

### *Types of electrode*

A full description of the manufacture, calibration and use of the different microelectrodes is again available in the account of a previous investigation (Boshier 1979). One electrode was double-barrelled (tip diameter 2  $\mu\text{m}$ ) one channel being a  $\text{K}^+$  sensitive electrode made from potassium liquid ion-exchanger (Corning 477317) and the other being a conventional electrode filled with 150 mM KCl for measuring the endocochlear potential. Such a double barrelled  $\text{K}^+$  sensitive microelectrode was essential to exclude artefacts arising from the spatial separation of the tips of single electrodes. Longitudinal variations in the endocochlear potential exist and a 1 mV difference between single electrodes would be equivalent to a  $\text{K}^+$  concentration change of 5.6 mM at the endolymph concentration. The use of 150 mM KCl as a filling solution prevented the occurrence of an electrode tip junction potential in endolymph which would also have had a similar effect.

The other electrode was a Na sensitive microelectrode of the recessed-tip type (tip diameter 5  $\mu\text{m}$ ) constructed with a 200  $\mu\text{m}$ -long cone of NAS 11-18 glass (Microelectrodes Inc.). These electrodes recorded potential changes accurately and gave linear responses in endolymph characteristics which previous workers had failed to achieve with their relatively short cones of Na sensitive

glass in inverted-tip type electrodes. Because a 1 mV error in the reading is only equivalent to a Na<sup>+</sup> concentration change of 0.04 mM at the endolymph concentration, spatial separation of the tips of the electrodes was not a serious problem. In a subsidiary series of experiments, single pH microelectrodes of the recessed-tip type (tip diameter 2.5 µm) made from H<sup>+</sup>-sensitive glass (Corning 0150) were used in place of the Na<sup>+</sup>-sensitive electrodes.

Repeated measurements of the calibrating solutions always gave identical readings and a better estimate of the sensitivity of the method was therefore required. All potentials were recorded to  $\pm 50$  µV. For the Na<sup>+</sup>-sensitive microelectrode this was equivalent to  $\pm 0.002$  mM at the 1 mM level and  $\pm 0.07$  mM at the 10 mM level. The corresponding figures for K<sup>+</sup> were  $\pm 0.28$  mM at 150 mM and for pH  $\pm 0.001$  unit at pH 7.4. The results are routinely expressed in the form: mean  $\pm$  standard error of mean, number of experiments (unless this is given in the adjacent text). The values give ionic concentrations and not activities to enable comparison with other findings to be made more easily.

#### Permeability and flux calculations

Due to the absence of a suitable direct method permeability alterations were quantified by means of the principle introduced by Hodgkin & Katz (1949) and developed for the cochlea by Johnstone and his colleagues (Johnstone 1970; Sellick & Bock 1974; Sellick & Johnstone 1975). In essence the rate of change in the endolymphatic concentration of the ion concerned for any selected moment during terminal anoxia, normalised with respect to the relevant electrochemical gradient is used to calculate the conductance of the ion at that moment according to the equation

$$LG = \frac{\Delta I}{\frac{RT}{zF} \ln \frac{a'}{a} + \Delta E}$$

where  $R$  is the gas constant,  $T$  is the absolute

temperature,  $z$  is the valency of the ion,  $F$  is Faraday's constant,  $a'$  and  $a$  are the activities of the ion in endolymph and perilymph respectively,  $\Delta E$  is the electrical potential difference between endolymph and perilymph,  $\Delta I$  is the rate of change in the endolymphatic concentration of the ion,  $G$  is the true conductance and  $L$  is a constant. Although  $L$  is specific for any particular animal species, its value is not known precisely and the term  $LG_i$  is taken as an index of the permeability of the ion which enables relative permeabilities and permeability variations to be determined accurately. In anoxia its value remains unchanged for 3–6 min after the minimum peak in the endocochlear potential following which substantial increases in cation permeability occur. The cationic conductances during this short stable period have been shown to be representative of the pre-anoxic conditions (Bosher 1979).

When multiplied by the appropriate ionic driving force, conductances determined in this way provide an estimate of the rate of concentration change due to the passive forces at any time during the experiment. Thus the contribution of the active transport processes can be derived from the net concentration change according to the equations

$$cJ_{\text{pass}} = LG_i \left( RT \ln \frac{a'}{a} + \frac{EP}{z} \right)$$

$$cJ_{\text{act}} = cJ_{\text{net}} - cJ_{\text{pass}}$$

where  $J_{\text{active}}$ ,  $J_{\text{net}}$  and  $J_{\text{pass}}$  are the active, net and passive ionic fluxes at time  $t$ ,  $c$  is the volume area constant for the endolymph system of the animal (so  $cJ$  is the rate of concentration change) and  $EP$  is the endocochlear potential. While the true fluxes ( $J$ ) cannot be specified because  $c$  has not been measured with sufficient reliability, the results do enable the various ionic fluxes to be compared with each other and the changes in the fluxes with time can be followed.



Table I Normal endolymph composition

|                             | Range       | Mean S.E.M    | No of experiments |
|-----------------------------|-------------|---------------|-------------------|
| <i>Rat</i>                  |             |               |                   |
| Endocochlear potential (mV) | 79.2–97.0   | 87.1 ± 1.0    | 35                |
| Endolymph                   |             |               |                   |
| [Na <sup>+</sup> ] (mM)     | 0.45–1.30   | 0.76 ± 0.05   | 20                |
| [K <sup>+</sup> ] (mM)      | 137.4–167.1 | 152.2 ± 2.6   | 15                |
| pH                          | 7.430–7.440 | 7.436 ± 0.001 | 5                 |
| <i>Guinea pig</i>           |             |               |                   |
| Endocochlear potential (mV) | 81.1–89.5   | 83.6 ± 0      | 4                 |
| Endolymph                   |             |               |                   |
| [Na <sup>+</sup> ] (mM)     | 0.79–1.36   | 1.1 ± 0.12    | 4                 |
| [K <sup>+</sup> ] (mM)      | 165.8–168.6 | 167.2 ± 1.4   | 4                 |

The guinea pig results all lie in the upper portion of the normal range found in a similar but more extensive previous study (Bosher 1979)

## RESULTS

### Control findings

The values of the endocochlear potential, the endolymphatic Na<sup>+</sup> and K<sup>+</sup> concentrations and the endolymphatic pH found during the preliminary control periods are given in Table I. They do not differ from the normal results obtained previously in this laboratory (Bosher 1979) and are in good accord with the levels now generally accepted. The pH figures confirm the original report of Misrahy et al. (1958). Owing to the technical difficulties involved in simultaneous perilymphatic measurements were not made. However, these are not significantly altered by the administration of ethacrynic acid (Bosher et al. 1973; Melichar & Syka 1978) and the values determined separately in normal animals (Bosher 1979) were used in the calculations. For Na<sup>+</sup> these were 131.7 mM in the rat and 151.7 mM in the guinea pig; for K<sup>+</sup> they were 6.8 mM and 3.8 mM respectively.

### Endolymphatic changes after ethacrynic acid (rat)

A dose of 60 mg kg<sup>-1</sup> ethacrynic acid was given intravenously because it is the smallest which produces complete abolition of the positive component of the endocochlear potential in the rat (Bosher et al. 1973). Potential recordings were obtained in 35 animals; Na<sup>+</sup> results were satisfactory in 20 of these and K<sup>+</sup> results in 15 (combined results were available for 9).

The endocochlear potential (Fig. 1) began to decrease after a latent period of 2.4(±0.3) min. Its rate of fall was slower and much more variable than in anoxia, the greatest negative potential being attained between 9 and 39 min. The magnitude of this negative potential was time-dependent, values before 15 min were of the same order as those in anoxia but they declined by about half during the next 10 min and then remained at the same level (Table II). Recovery began after 1 to 7 min. Once

Table II The time-dependence of the minimum endocochlear potential after ethacrynic acid (60 mg kg<sup>-1</sup> i.v.) in the rat

|                                     | <16   | 16–20 | 1–5   | >25   |
|-------------------------------------|-------|-------|-------|-------|
| Min. after injection                |       |       |       |       |
| Minimum endocochlear potential (mV) | -39.1 | -35.1 | -26.7 | -21.9 |
| Mean                                | ± 1.7 | ± 1.7 | ± 3.5 | ± 3.5 |
| S.E.M.                              | 12    | 12    | 6     | 5     |
| Number of animals                   |       |       |       |       |

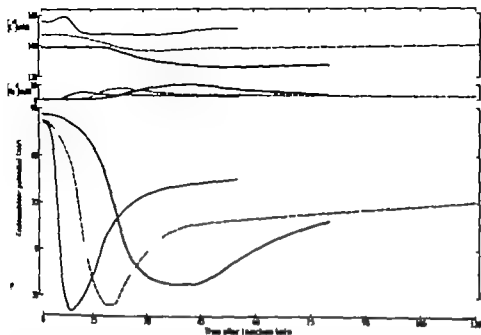


Fig. 1 Representative experiments showing the variation in the endolymphatic changes produced by an intravenous injection of 60 mg/kg ethacrynic acid in the rat. The magnitude of the negative peak in the endocochlear potential decreased as the dose of its occurrence after the injection increased (Table II). The maximum elevation of the endolymphatic Na<sup>+</sup> concentration was always found a few minutes after the peak in the potential. When

the potential began to decline the Na<sup>+</sup> concentration began to rise, although this is not evident because of the scale used. The different patterns of the effects upon the endolymphatic K<sup>+</sup> concentration are illustrated. The experiments were terminated after progressively longer intervals by the induction of anaesthesia (alterations not shown).

established. It was rapid and approximately linear for 6.1 ( $\pm 1.0$ ) min when the rate progressively diminished to a slow constant value only 2–8% of that during the initial phase. As a result the endocochlear potential was still 47.0–60.7 mV below the individual control levels at 120 min ( $n=3$ ).

The endolymphatic Na<sup>+</sup> concentration began to rise at the same time as the endocochlear potential began to decrease, although this is not evident in Fig. 1 because of its small scale. After the negative peak in the potential the rate of increase in the concentration rapidly slowed and then the concentration commenced to decrease so that a maximum of 8.15 ( $\pm 0.51$ ) mM was produced just after the minimum in the potential. The rate of the return of the Na<sup>+</sup> concentration towards normal progressively decreased with time and the

concentration was 0.72–1.65 mM above the initial levels at 120 min ( $n=3$ ).

The alterations in the endolymphatic K<sup>+</sup> concentration were less consistent in nature. In 14 animals there was an overall decrease in the concentration but in 5 of these the decrease was preceded by a transient increase of 1.2–3.4 mM maximal at 6 or 7 min and in the remainder the onset of the decline lagged behind the other changes by up to 5 min (Fig. 1). The total fall in concentration was 5.6–12.9 mM and subsequently the concentration rose again so slowly that the levels at 120 min were still 6.9–9.3 mM below the control values ( $n=3$ ). In the remaining animal there was no variation in the K<sup>+</sup> concentration during the 14 min it was followed.

The effects upon the endolymphatic pH were observed in 5 rats. Immediately after the

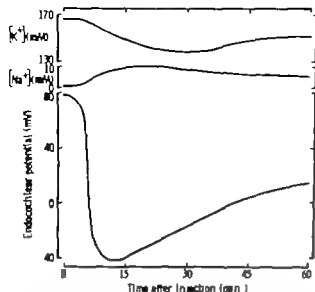


Fig. 2 The typical effects produced by intravenous ethacrynic acid ( $40 \text{ mg kg}^{-1}$ ) in the guinea pig. The latent period was 1 min and the positive component of the endocochlear potential was completely abolished for 1 min. Although not apparent at this scale the endolymphatic Na<sup>+</sup> concentration began to rise when the potential commenced to decline. The endolymphatic K<sup>+</sup> concentration remained unchanged for 3 min. The differences in scale from Fig. 1 will be noted.

injection there were differing and transient alterations in three experiments but no change in the remaining two. Thereafter the pH remained constant until the experiments were terminated at 100 min. The interpretation of the early changes is difficult. The ethacrynic acid administration often resulted in short-lived alterations in respiration (combinations of stimulation, depression and irregularity) and its influence upon the blood pH is conjectural. However, there was no consistent pattern about the initial action upon the endolymphatic pH and the conclusion that it was not directly affected by ethacrynic acid seems reasonable in view of the subsequent invariant level.

#### *Endolymphatic changes after ethacrynic acid (guinea pig)*

The effects of ethacrynic acid were followed in 4 guinea pigs for 60 min to determine whether any significant differences from the rat occurred, since the results of perilymphatic

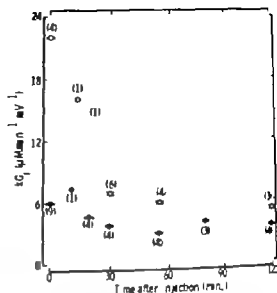


Fig. 3 The action of intravenous ethacrynic acid ( $40 \text{ mg kg}^{-1}$ ) upon the gross permeability of the endolymphatic membranes in the rat, calculated as described in the text. For the purposes of determining the effect of the drug upon active transport, the decrease in both the Na<sup>+</sup> (O) and the K<sup>+</sup> (□) permeabilities was considered to be progressive. The significance of the solitary Na<sup>+</sup> finding at 11 min was uncertain because of the technical difficulties experienced at this time. However, the active transport results subsequently suggested that a transient increase in Na<sup>+</sup> permeability did occur. Each point indicates a mean and S.E.M. for the number of experiments specified in parentheses. The zero time values are the same levels taken from Boshier, 1979.

perfusion with ethacrynic acid were not the same in the two species (Boshier, 1979). Preliminary experiments revealed that the smallest intravenous dose of the drug which completely abolished the positive component of the endocochlear potential (i.e. which was equivalent to the dose given in the rat) was  $40 \text{ mg kg}^{-1}$  and this was the dose administered. After a latent period of 1 min, the endocochlear potential fell to a minimum of  $-39.5 (\pm 2.8) \text{ mV}$  at  $12.8 (\pm 1.4) \text{ min}$ , a value which is not significantly different from the anoxic values as would be expected from the time relationship. Recovery of the potential commenced after 1 to 2 min and was biphasic in character. The endolymphatic Na<sup>+</sup> concentration increased by  $10.7 (\pm 1.2) \text{ mM}$  and the K<sup>+</sup> concentration decreased by  $34.1 (\pm 5.1) \text{ mM}$  before both began returning towards normal. Compared with the rat, recovery was somewhat

in the case of Na but faster in the case of K. As described below these findings (Fig. 7) indicate that ethacrynic acid given intravenously has essentially the same actions in the two species, the differences found being only of a minor quantitative nature.

### Permeability alterations

Each experiment was terminated with anoxia induced at varying times after the initial injection and the relative alterations in the permeabilities of the endolymphatic membranes which had arisen were calculated as described earlier. The results (Fig. 3) showed that the ethacrynic acid had produced decreases of  $44 \pm 4\%$  in the Na permeability ( $n=10$ ) and  $74 \pm 3\%$  in the K permeability ( $n=9$ ) in the rat. These permeability effects were present at 30 min and were long-lasting: no evidence of recovery being present at 120 min. Attempts to determine the time course of the reductions more precisely were unsuccessful. This was because some ethacrynic acid probably remained in the inner ear tissues when anoxia was induced shortly after the drug's administration. In these circumstances the permeability changes would eventually develop fully during the anoxic period due to its continued action. It will be noted that these permeability effects proved to be different in type to those expected from our preliminary investigation (Bosher et al. 1973). An advantage of the dose of ethacrynic acid selected was that the rise in the endolymphatic Na concentration was well below the level of 12 mM. This is the concentration which is associated with a marked increase in the permeability of the endolymphatic membranes to cations (Bosher 1979). The complications of a secondary change of this type were therefore completely avoided. The guinea pig experiments were too few for the total range of the effects to be determined accurately. But the mean reductions found in the Na and K permeabilities were 45% and 68% respectively (i.e. about the same as in the rat).

### Effect on active transport

The changes in the endolymphatic ion concentrations provide very little information by themselves about the effect of ethacrynic acid upon the active transport mechanisms. The marked alterations in the endocochlear potential produce equally marked alterations in the passive fluxes and these must be taken into account first. However this can be done as described earlier and the action upon the ion-transporting mechanisms is then revealed.

In every animal the active processes responsible for Na and K transport and endocochlear potential production were completely abolished by the time of the minimum peak in the potential. A problem arises about the exact interpretation of the details of this action. As explained above it was not possible to determine experimentally the precise time courses at the beginning of the permeability effects. So the initial time courses illustrated in Fig. 3 were used in the calculations. Although these were undoubtedly good first approximations the early differences between the various processes apparent in Fig. 4 might have been due to small variations in the true time courses of the permeability effects rather than actual divergences between the processes themselves. For example the initial small rise in Na activation might have been due to a slight transient increase in Na permeability before it declined to its later level (Fig. 3). Whatever their cause these initial differences were relatively minor in nature and activation was reduced to zero at the time of the minimum endocochlear potential in every case. Consequently the principal effect of the ethacrynic acid in this respect was to fully inhibit all the transport processes measured at about the same overall rate.

At and after the time of complete inhibition the reductions in permeability measured individually could be used in each experiment. Thus similar problems do not arise about the findings in this period. These revealed that the Na transport increased much more quickly

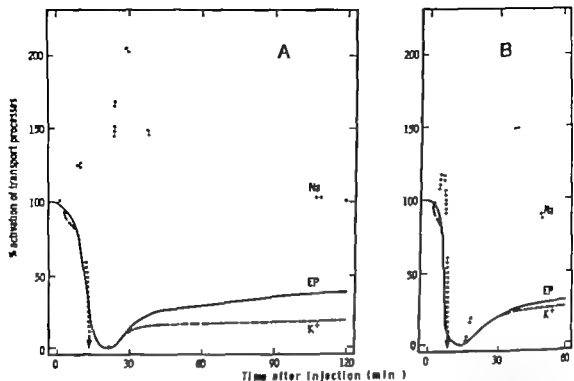


Fig. 4 The action of intravenous ethacrynic acid upon the active transport processes responsible for maintaining the endolymphatic composition in the rat (A) and guinea pig (B) calculated as described in the text. The

representative experiments (illustrated are the one depicted in Fig. 1 (170 min animal) and Fig. 2). The port rates have been normalised with respect to the during the control period (100 %)

ly and to a much higher level of activation than the  $K^+$  transport or the potential production during the early part of the recovery period. Then at  $26(\pm 2)$  min in the rat there was a sudden and substantial decrease in the rate of  $Na^+$  transport which had reached an activation level of  $241(\pm 19)\%$ . At the same time the obvious later decline in the rates of  $K^+$  transport and potential production commenced after their previously progressive increases to activation levels of  $18(\pm 3)\%$  and  $20(\pm 3)\%$  respectively. This is not clearly visible in Fig. 4 because of its small scale. Thereafter the increase in the rates of recovery of the  $K^+$  transport and the potential production were very slow. This is the reason why complete functional recovery after a single intravenous dose of ethacrynic acid takes up to 10 hours (Bosher et al. 1976; Brummett et al. 1977).

It must be stressed that the results represent alterations in the activation levels of the processes concerned. The average ratio of the

amount of  $K^+$  transported to the amount  $Na^+$  transported during the control period was 13:1 in the rat. At the time of the peak the  $Na^+$  activation values, this ratio had fallen to 1:1 but had increased to 2.5:1 at 170 min. Thus the amount of  $K^+$  transported always exceeded the amount of  $Na^+$  except for the brief period when they were equal. The findings in the guinea pig studies demonstrated that the effects of intravenous ethacrynic acid were qualitatively the same as in the rat although quantitative differences were present (Fig. 4). Detailed presentation of these differences does not seem justified because of the small number of experiments performed. But for example the ratio between active  $K^+$  and  $Na^+$  transport in the control period was 8:1 compared with 13:1 and the sudden decrease in the recovery rates occurred at 35 min compared with 26 min.

From the foregoing description it follows that the unchanged levels of the endolymphatic  $K^+$  concentration which have been de-

scribed previously in the guinea pig and cat (Bruslow & Gordon 1973; Silverstein & Yoles 1971) were due to an equivalent effect upon both the passive and the active fluxes. This was certainly the case in the one rat exhibiting this phenomenon in the present investigation where the inhibition of the transport processes followed its usual course and the reduction in the passive  $K^+$  flux precisely paralleled the reduction in the active  $K^+$  transport.

## DISCUSSION

### *The nature of the ototoxic action of ethacrynic acid*

The present investigation has confirmed that ethacrynic acid has three main toxic effects upon the endolymph system and it has been possible to obtain measurements of their respective magnitudes. One of the effects is inhibition of the stria transport mechanisms concerned with endolymphatic homeostasis. The underlying enzymic processes seem to have much the same sensitivity to ethacrynic acid in the initial stages and thus would be expected if the primary action of the drug was upon a regulatory adenylate cyclase as suggested by Palocher & Thalmann (1977). However the marked differences in ion transport which developed during the recovery phase are less easy to explain on this basis. A direct action upon the transporting enzymes therefore remains a distinct possibility and the results do not help in distinguishing between the two alternative concepts.

One feature which has been confirmed is the existence of a relatively long latent period before any change occurs in the active transport. The latent period after an equivalent dose of furosemide administered by the same route is less than one minute (Bosher unpublished observations) so the method of administration and access to the stria cells do not appear to be important factors. Delay due to chemical transformation of the drug, say into a thiol adduct, has been proposed but

seems to be excluded by the findings of Fox & Brummett (1974a, b). In consequence the nature of the molecular interactions responsible for the latent period remains unknown.

The second major effect which has been documented is the decrease in the overall permeability of the endolymphatic membranes. This pursues a quite different time course from the enzyme inhibition developing over 30 min and showing no signs of recovery at 120 min. Similar reductions in ionic permeability occur in the kidney (Burg & Green 1973) but the maximum decrease there has only been by about 70% i.e. considerably less than in the ear. Another unusual aspect of the endolymphatic changes is the preferential diminution in the  $K^+$  permeability. The situation accordingly suggests that ethacrynic acid might act upon some specialised region of the boundary membranes. Nevertheless more direct evidence of this is lacking. Whatever their site the decreases in permeability will tend to reduce the magnitude of the passive cation movements. Thus the recovering transport mechanisms will be able to produce larger increases in the endolymphatic concentrations than if these passive movements were normal. The concentration alterations alone therefore greatly overestimate the degree of recovery of the transport processes.

The last of the principal effects delineated is the substantial reduction in the rate of recovery starting at about 26 min in the rat and a few minutes later in the guinea pig. At this time the cation concentrations have returned less than halfway toward the control values and their rates of change are comparatively slow. The diminution in the active transport, consequently is most unlikely to be the result merely of a reduction in enzyme activation secondary to the alterations in the ionic concentrations particularly in view of the severity and rapidity of the fall in the  $Na^+$  transport. A well-known action of ethacrynic acid is its depression of oxidative metabolism and ATP production which often does not become

manifest in other tissues for some time after its administration (Rossum & Ernst 1978). It therefore seems reasonable to propose that a delayed decrease in stria ATP production is responsible for the delayed decrease in active stria transport, the cause of the excessively prolonged recovery described previously (Boshier et al. 1976; Brummett et al. 1977). The possibility of the initial inhibition of active transport being due to diminution in the stria energy supply is completely excluded by the results of Kusakari et al. (1978). An increase in arterial oxygen tension has been reported to protect the cochlea partially against the toxic effects of ethacrynic acid but whether this is brought about by some action on cochlear oxidative metabolism has yet to be determined (Prazma & Pecorak 1975).

The present investigation has been concerned with the acute functional changes in the endolymph system after a single intravenous dose of the drug. It has also provided a unique opportunity to correlate these with the morphological alterations in the stria vascularis. Systematic ultrastructural examination of typical animals at frequent intervals was therefore undertaken and the results will be presented in a subsequent paper. Other problems concerning the ototoxic action of ethacrynic acid remain to be resolved such as the consequences of the new pathological features which appear in the stria vascularis after 12 hours or more (Boshier et al. 1976; Brummett et al. 1977), the aetiology of the disastrous cumulative effects of small daily doses (Crifó 1973) and the basis of the potentiation by aminoglycoside antibiotics specific to the inner ear (Fox & Brummett 1979).

#### *The normal processes controlling the endolymphatic composition*

The endocochlear potential is now generally believed to consist of two main components. The first is a positive electrogenic potential usually attributed to active  $K^+$  transport although other possibilities could exist (e.g. proton transport). Direct experimental evidence

is scanty and consists essentially of the discovery of a correlation between the level of the endocochlear potential and the stria  $K^+$  transport when the endolymphatic  $K^+$  concentration is decreased by perfusion (Selick & Bock 1974). In the studies reported here a similar correlation has now been found in a second situation namely after the administration of ethacrynic acid. As described above is a reasonably close correspondence between the activation levels of the potential-producing and  $K^+$  transporting processes occurred throughout the recovery period.

It is true that a consistent small discrepancy was present: the potential-producing processes always appearing slightly more active than the  $K^+$  transporting ones. There are two possible explanations for this. Firstly ethacrynic acid is known to increase the trans-epithelial electrical resistance (e.g. Burg & Green 1973) so the transport mechanisms could produce a higher potential for any given level of activation than in normal circumstances. The consequent overestimation of the recovery of these processes would tend to result then in the discrepancy under consideration. Alternatively the discrepancy might indicate the existence of another subsidiary source for the positive electrogenic potential. At present it is impossible to determine which possibility is correct in the absence of satisfactory direct methods of investigation. Nevertheless the discrepancy is only a small one and there seems no doubt that active  $K^+$  transport is the major source of the positive component of the endocochlear potential.

The other principal component is the negative potential found after the positive component is abolished by anoxia or inhibitors. It has been established that this negative component behaves quantitatively as a modified  $K^+$  diffusion potential which is dependent upon the measured selective permeability of the endolymphatic system to  $K^+$  and upon the  $K^+$  and  $Na^+$  concentration gradients between endolymph and perilymph apart from a minor contribution due to the remaining ions (Bosher

779) However the location and extent of the biophysical component of the endolymphic membranes responsible for the overall selective permeability has yet to be determined. A possible third component of the endocochlear potential has been described in the guinea pig (Sellick & Johnstone 1974 Kusakari & Thalmann 1976 Kusakari et al. 1978) it has been shown to be completely absent in the rat (Bosher 1979). Consequently it does not complicate the interpretation of the experiments on this species reported here.

In the 9 rat experiments in which simultaneous Na and K results were obtained the mean diffusion potential calculated from the standard Hodgkin & Katz (1949) equation and the measured values at the time of the minimum potential peak was  $-20.0 (\pm 4.4)$  mV. The average endocochlear potential actually found was  $-24.7 (\pm 3.9)$  mV, the discrepancy of  $4.7 (\pm 0.9)$  mV being identical with the one described in other situations (Bosher 1979). The difference in the value of this negative peak to the one found after anoxia in normal animals is due to the decrease in the relative permeability of the endolymph system to K after ethacrynic acid; the measured Na/K permeability ratio at this time being  $0.63 (\pm 0.08)$  compared with the normal 0.27 in the rat.

Finally the results also provide evidence about the relationship between the mechanisms responsible for cation transport. As reported, the normal ratio of active K to Na transport is 13:1 in the rat and 8:1 in the guinea pig. Although the latter value is less than the 66:1 derived theoretically for the guinea pig by Sellick & Johnstone (1975) it is still well outside the coupling ratios of the Na/K-activated ATPases. In addition the large differences in the active transport systems demonstrated during the recovery period indicate that most of the K transport must be independent of the Na transport. These findings consequently provide further experimental evidence in support of the view that some enzyme other than a Na/K-activated

ATPase is directly responsible for the potential generation and at least most of the K transport (Kujpers & Bonting 1970b Sellick & Bock 1974 Sellick & Johnstone 1975 Paloheimo & Thalmann 1977 Kusakari et al. 1978). It follows from this that the K transport will be largely and possibly completely electrogenic in nature and this is confirmed by the close association between the K transporting and potential-producing systems described in the recovery period.

The importance of Na/K-activated ATPase in the endolymph system has not been in question since the initial studies of the effects of ouabain (Konishi & Mendelsohn 1970; Kujpers & Bonting 1970a). Moreover it is present in the stria vascularis in a high concentration (Kujpers 1974 Kujpers & Bonting, 1969 Matschinsky & Thalmann 1970) but it cannot be concerned directly with endolymphatic cation transport. Instead preliminary results in this laboratory (Bosher unpublished observations) suggest its role to be an indirect one such as maintaining the internal ionic environment of the transporting cells.

## ACKNOWLEDGEMENT

I am grateful to the Department of Medical Illustration, The Middlesex Hospital for preparing the diagrams.

## ZUSAMMENFASSUNG

Endolymphatische Veränderungen infolge intravenöser Injektion von Ethacrynsäure an Ratten, 60 mg kg<sup>-1</sup> war den bis zu 120 Minuten lang beobachtet unter Verwendung konventioneller und ionenselektiver (Na<sup>+</sup>, K<sup>+</sup> und pH) Mikroelektroden. Man fand als Ursache 3 deutliche Wirkungen auf das Endolymphsystem. Zunächst wurden die strahlen potential-produzierenden und kationen-transportierenden Prozesse total gestoppt. Wiederherstellung begann schnell und war zunächst rapide. Dann verlangsamte sie sich erheblich, vermutlich infolge der Abnahme der strahlen Energieproduktion mit zunehmendem Beginn und langer Dauer. Gleichzeitig mit diesen Wirkungen auf die aktive Transportion trat eine Verkleinerung der gesamten Kationenpermeabilität des Endolymphsystems auf. Dort hatte einen verschiedenartigen Zeitablauf und betraf K<sup>+</sup> erheblich mehr als Na<sup>+</sup>. Die Befunde geben außerdem weitere Informationen über den Mechanismus, der für die normale Beschaffung



der Endolympe verantwortlich ist. Qualitativ ähnliche Resultate wurden bei Experimenten mit Meerschweinchen gefunden.

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## SPEECH DISCRIMINATION OF PATIENTS WITH HIGH FREQUENCY HEARING LOSS

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(Received March 7 1979)

**Abstract.** Mean speech discrimination curves were measured in three groups of patients having severe, moderate and high-tone hearing losses in pure-tone audiograms, 20 patients being tested in each group. The audiograms sloped linearly by 22 dB/oct. Recruiting ears in Reger's test were excluded. The group with moderate hearing loss achieved significantly poorer discrimination scores at high sound pressure levels than the group with severe hearing loss, a phenomenon not reported before. However, the results agree with preceding work in which equivalent sensorineural hearing losses were simulated with filter equipment. The causes of the phenomenon and significance for hearing aid selection are discussed.

Clinical experience has often given the impression that some patients with relative serious low-pass hearing loss can utilize the remnants of their hearing better than some with moderate but in other respects similar hearing loss. In an earlier experiment made to examine this phenomenon, three degrees of low-pass hearing loss were simulated using a filter apparatus and the corresponding discrimination curves measured using young subjects with normal hearing (Krukaanniemi 1979a). It was shown that theoretically there really are some significantly disadvantageous high frequency hearing losses which may yield markedly poorer discrimination scores at high sound pressure levels than other more serious hearing losses of the same type.

One case of this kind proved to be a linearly decreasing hearing loss of 22 dB/octave beginning at about 250 Hz. The discrimination curve for this hearing loss crossed the curve yielded by a parallel hearing loss beginning at 125 Hz. The crossover point was 44 dB re

$2 \times 10^{-9}$  N/m<sup>2</sup> and 75% discrimination. This phenomenon has not been reported before in the literature and it encouraged the author to attempt a measurement of the discrimination curves of patients having an equivalent clinical high frequency hearing loss. A further indication for this study was the lack of earlier results concerning the discrimination ability of Finnish-speaking patients with various grades of high frequency hearing loss.

## MATERIAL AND METHODS

72 patients whose pure-tone audiogram showed typical high frequency hearing loss were selected for further examination. An anamnesis was taken in order to outline 1) etiology of the hearing loss, 2) use of a hearing aid or other auxiliary facilities, 3) the patient's subjective opinion on his/her difficulties in daily life.

The ear status and pure-tone threshold of the patients were examined and those showing recruitment in Reger's test were excluded so as to achieve a loudness function and its influence on the threshold curve equal to the preceding experimental study. The elimination of recruiting ears may increase the relative number of retrocochlear losses in the present material. The preceding experimental study with filtered words using normal-hearing subjects gave a reason to anticipate a very disadvantageous high frequency hearing loss among non-recruiting ears. Discharging ears were similarly eliminated from the subsequent tests.

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$2 \times 10^{-5} \text{ N/m}^2$  and 75% discrimination. This phenomenon has not been reported before in the literature and it encouraged the author to attempt a measurement of the discrimination curves of patients having an equivalent clinical high frequency hearing loss. A further indication for this study was the lack of earlier results concerning the discrimination ability of Finnish-speaking patients with various grades of high frequency hearing loss.

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## MATERIAL AND METHODS

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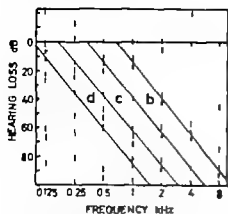


Fig. 1 The pure-tone audiograms for the patients tested in groups B, C and D were located in the areas marked b, c and d respectively. The slope of the audiograms is 2 dB/octave.

Ten cases of occupational hearing loss were excluded from the subsequent tests because of showing recruitment in Reger's test. Two cases were excluded because of acute discharging.

The aetiological factors involved in the 60 cases studied here are given in Table I. Impulse noise (pressure hammer rifle shots etc.) was the main cause (63%) amongst the patients having occupational high frequency hearing loss selected here; only one third of them (38%) using ear plugs regularly. All the patients used a hearing aid. 66% controlled the output levels themselves, but 30% of these adjusted the output level or compressor control wrongly in the opinion of an experienced hearing aid acoustician. When the patients were asked to select a hearing aid from an

Table II Discrimination scores for groups C and D at six sound pressure levels and significance of the differences in the Student's *t*-test.

| Output level (dB) SPL | Group B Discr % $\pm$ S.D. | Group C Discr % $\pm$ S.P. | Significance in Student's <i>t</i> -test |
|-----------------------|----------------------------|----------------------------|--|
| 57                    | 2.0 $\pm$ 1.6              | 17.1 $\pm$ 4.3             | $p < 0.001$                              |
| 67                    | 16.2 $\pm$ 4.0             | 37.3 $\pm$ 7.0             | $p < 0.001$                              |
| 77                    | 29.9 $\pm$ 6.2             | 47.0 $\pm$ 8.1             | $p < 0.001$                              |
| 87                    | 46.8 $\pm$ 7.0             | 51.3 $\pm$ 8.4             | $p < 0.1$                                |
| 97                    | 56.5 $\pm$ 6.5             | 50 $\pm$ 7.8               | $p < 0.01$                               |
| 107                   | 63.0 $\pm$ 7.5             | 47.5 $\pm$ 8.2             | $p < 0.001$                              |

assortment of 15 different devices in a session guided by the same hearing aid acoustician, 94% of them subjectively chose one with high frequency emphasis; the amplification beginning at 300–500 Hz.

52% of the total group of 60 patients claimed that group conversation caused them most inconvenience, while the rest suffered most inconvenience when using the telephone (26%) or because of the subjective distortion (breaking up) of sounds (22%).

The preliminary conversation screening and examination reduced the first group of 72 patients to 60, who were then divided into three groups of 20 according to their degree of hearing loss in a pure tone audiogram. These groups B, C and D occupy the areas marked b, c and d respectively in the pure tone au-

Table I Aetiological factors in high tone hearing loss found in the present study.

|  | Number of cases | %   |
|--|-----------------|-----|
| Acoustic trauma, occupational hearing loss     | 25              | 42  |
| Perinatal factors (asphyxia, maternal toxemia) | 20              | 33  |
| Ear infections later in life                   | 7               | 12  |
| Viral infections                               | 5               | 8   |
| Bacterial meningitis                           |                 | 3   |
| Uncertain vascular etiology                    | 1               | 2   |
| Total  | 60              | 100 |

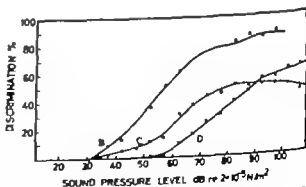


Fig. 2 Mean discrimination curves for three groups (B, C, D) with high-tone hearing loss corresponding to audiograms within the areas b and d in Fig. 1.

Table III The effect of presbycusis on discrimination of low-pass filtered speech in age groups 40.1 (I) and 50.3 (II) years

| Signal level<br>dB SPL | Filteration d                 |                 | Filteration                   |                  |
|------------------------|-------------------------------|-----------------|-------------------------------|------------------|
|                        | Group I<br>Discr % $\pm$ S.D. | Group II        | Group I<br>Discr % $\pm$ S.D. | Group II         |
| 10                     | 10.1 $\pm$ 5                  | 10.0 $\pm$ 5.0* | 43.0 $\pm$ 6.9                | 43.0 $\pm$ 6.0*  |
| 15                     | 52.3 $\pm$ 6...               | 51.0 $\pm$ 5.0* | 63.0 $\pm$ 6.1                | 63... $\pm$ 5.9* |
| 20                     | 79.1 $\pm$ 7.3                | 77.0 $\pm$ 6.9* | 73.1 $\pm$ 7.8                | 73.9 $\pm$ 6.6   |
| 25                     | 85.6 $\pm$ 8                  | 84.0 $\pm$ 8.0* | 77.0 $\pm$ 7.8                | 75.4 $\pm$ 7.1   |

\*  $p > 0.05$  Student's  $t$ -test.

ograms in Fig. 1. The mean ages for the groups were 54.9 (B) 51.3 (C) and 42.0 (D) years. Each patient was guided to a sound-proof room where 10 lists of 25 recorded PB words (Palva 1957 Jauhainen 1974) were presented to him monaurally with masking of the opposite ear where needed. The earphones used were MX 41/AR TDH 39 and the tape recorder a Revox A77. The sound pressure level being monitored using a Madsen OB 60 audiometer. The words were presented first at a level 40 dB above the speech reception threshold (SRT) of the patient established roughly by the method described by Palva (1952). The next list was then presented at a level 5 dB lower and so on until the discrimination score was 10% or less. The background noise was lower than 45 dB (A). The patient repeated the words and was encouraged to guess if he was not sure about the given word for the erroneous answers being written down for further investigation. The average discrimination scores for each test group were calculated in 5 dB steps from 37 to 11 dB re  $2 \times 10^{-9}$  N/m<sup>2</sup>.

## RESULTS

The average discrimination curves for the groups B, C and D are seen in Fig. 2, in which the difference between the scores of groups C and D at very high and rather low sound pressure levels are statistically significant (Student's  $t$ -test,  $p < 0.001$ ) as seen in Table II.

## DISCUSSION

The present findings corroborate those of the simulation experiments carried out on subjects with normal hearing (Kiikkaanniemi 1979a) that is there also seems to exist a real clinical group of patients (C) who have a weaker discrimination ability than any other group (D) with a poorer pure-tone audiogram. The pure tone threshold of the group C begins to decrease linearly by 22 dB/octave at the frequency range 200–300 Hz, and the discrimination curves of C and D cross at the point 90 dB re  $2 \times 10^{-9}$  N/m<sup>2</sup> and 51% discrimination, the curve for group C failing to rise significantly above this level. Since the ears showing recruitment in Reger's test were excluded, the distortion associated with cochlear signal processing was probably small.

The difference in the mean ages between the groups C and D was relatively large, 9.3 years. Palva & Jokinen (1970) found using the monaural and Matzker's binaural test that speech discrimination scores in the filtered speech test began to fall by the fourth decade and that there was marked asymmetry in the age groups over 60 years. Using masking noise (S/N ratio +22, +1, +7 and -3 dB) Jokinen (1973) also demonstrated an almost significant difference ( $p < 0.05$ ) in discrimination scores between the age groups 40–49 and 20–29 years but not between the groups 40–49 and 50–59 years. The filtered speech test may be more sensitive in revealing discrimination loss caused by presbycusis (Jokinen, 1973).

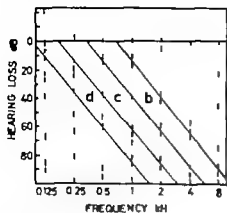


Fig. 1 The pure-tone audiograms for the patients tested in groups B, C and D were located in the areas marked b, c and d respectively. The slope of the audiograms is 2 dB/octave.

Ten cases of occupational hearing loss were excluded from the subsequent tests because of showing recruitment in Reger's test. Two cases were excluded because of acute discharging.

The aetiological factors involved in the 60 cases studied here are given in Table I. Impulse noise (pressure hammer rifle shots etc.) was the main cause (63%) amongst the patients having occupational high frequency hearing loss selected here; only one third of them (38%) using ear plugs regularly. All the patients used a hearing aid; 66% controlled the output levels themselves, but 30% of these adjusted the output level or compressor control wrongly, in the opinion of an experienced hearing aid acoustician. When the patients were asked to select a hearing aid from an

Table II Discrimination scores for groups C and D at six sound pressure levels and significance of the differences in the Student's *t*-test.

| Output level (dB) SPL | Group D Discrim. $\pm$ S.D. | Group C Discrim. $\pm$ S.P. | Significance in Student's <i>t</i> -test |
|-----------------------|-----------------------------|-----------------------------|--|
| 57                    | 7.0 $\pm$ 1.6               | 17.1 $\pm$ 4.3              | $p < 0.001$                              |
| 67                    | 16 $\pm$ 4.0                | 37.3 $\pm$ 7.0              | $p < 0.001$                              |
| 77                    | 29.9 $\pm$ 6.2              | 47.0 $\pm$ 8.1              | $p < 0.001$                              |
| 87                    | 46.8 $\pm$ 7.0              | 51.3 $\pm$ 8.4              | $p < 0.1$                                |
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assortment of 15 different devices in a session guided by the same hearing aid acoustician, 94% of them subjectively chose one with high frequency emphasis, the amplification beginning at 300–500 Hz.

52% of the total group of 60 patients claimed that group conversation caused them most inconvenience, while the rest suffered most inconvenience when using the telephone (26%) or because of the subjective distortion (breaking up) of sounds (22%).

The preliminary conversation screening and examination reduced the first group of 77 patients to 60, who were then divided into three groups of 20 according to their degree of hearing loss in a pure tone audiogram. These groups B, C and D occupy the areas marked b, c and d respectively in the pure-tone au-

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| Perinatal factors (asphyxia, maternal toxemia) | 70              | 33  |
| Ear infections later in life                   | 7               | 12  |
| Viral infections                               | 5               | 8   |
| Bacterial meningitis                           | 1               | 3   |
| Uncertain vascular etiology                    | 1               |     |
| Total  | 60              | 100 |

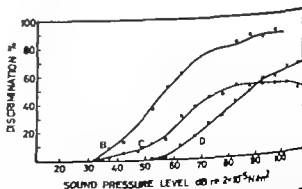


Fig. 2 Mean discrimination curves for three groups (B, C and D) with high-tone hearing loss corresponding to audiograms within the areas b, c and d in Fig. 1.

compared with that of the English speaker although there are large individual differences in speech spectra (Byrne 1977) the linguistic of the Finnish language may also add to the errors caused by upward masking of the second F in group C. Conversely however it also appears from the present results to be easier to utilize the remnants of one's hearing if one's audiogram belongs to groups B or D. Upward masking probably effectively eliminates the advantage which some Finnish patients (group C) with high frequency hearing loss have due to the fact that the speech power in their language is concentrated in the frequency area where their residual power of hearing lies.

The present results and those of the preceding experimental study suggest that the sloping frequency area where the disadvantageous masking effect is present is rather narrow about  $\frac{1}{2}$  octave. Nevertheless mild high frequency hearing losses are frequently located precisely in this area. These facts make it important in clinical practice to single out patients belonging to group C when selecting a hearing aid. The distorting masking noise of the first formant is best eliminated by a hearing aid in which amplification starts at 500 Hz. As seen here the patients in this group subjective ly choose such a hearing device because of its significantly better discrimination ability. Another possibility would be a device which would present the main frequency areas of F and F to opposite ears. Some patients however also show a third type of masking, central masking (Denaber & Pickett, 1974) which eliminates even the advantage of this kind of hearing aid. Also the exact separation is difficult, because F and F very often overlap.

### ZUSAMMENFASSUNG

Sprachverständnistests für drei Gruppen von Patienten mit einem schweren, mittelschweren und leichten Hörverlust wurden mit den finnischen PB-Wörtern getrieben. 20 Patienten wurden in jeder Gruppe untersucht. Das Audiogramm jedes Patienten auch parallel 22 dB/Oktave. Der Oberrand der drei positiven Rekrutierung an Reifer Tests wurden ausgeschlossen. Die Gruppen mit

Kürzungsversuche auf letzten Lautstärken als die Gruppe mit dem schweren Hörverlust - eine Erscheinung, die man nicht früher rapportiert hat. Die Resultate stimmen doch mit dem früheren Experimenten überein, wo die äquivalenten sensorischen Hörverluste mit einem Filterapparat simuliert wurden. Die Ursachen und die Bedeutung dieser Erscheinung für die Auswahl des Hörapparates wird diskutiert.

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Therefore two additional groups with mean ages of 50.3 and 40.1 years were retested in order to study the significance of presbycusis in the circumstances of the present study. Fifteen subjects were tested in each group simulating the hearing losses C and D using the filter equipment described earlier (Kiukaanniemi 1979a). The results in Table III show that the small differences caused by presbycusis cannot wholly explain the crossing of the discrimination curves C and D in the present study.

The discrimination curve for group II shows a plateau at the level 75–80 dB due to the differential sensitivity to high and low frequencies (Boothroyd 1967) as was also found in the experimental part of this study (Kiukaanniemi 1979a). In the present case the plateau is relatively small however and not of any great clinical importance and will thus not be discussed any further in this connection.

The crossing of the curves C and D on the other hand is worth further consideration. It has not been reported before although its major cause, the upward spreading of masking noise, has been mentioned by many authors (Jerger et al. 1960; Bilger & Hirsh 1956; Rittman 1962; Martin & Pickett 1970; Byrne 1977; Martin et al. 1972) and Danaher et al. (1973) have shown in experiments using synthetic vowels that when  $F_2$  was presented alone there was a marked difference in performance between the normal subjects and those with sensorineural hearing loss. A much greater difference was found when the subjects listened to  $F_2$  transitions in the presence of  $F_1$  energy. The performance of the subjects with sensorineural hearing loss was much poorer than that of the normal subjects although there was a marked variability. The existence of a variable masking effect of  $F_1$  on  $F_2$  discrimination depending on the degree of hearing loss as found in the present study would obviously explain the great variability in the performance of person with sensorineural hearing loss described by Danaher

& Pickett (1974). Altogether the poor performance of groups C may be of importance for hearing aid evaluation in the case of such patients. The poor performance is mainly a result of three factors: 1) the emphasized masking effect of  $F_1$  on  $F_2$  discrimination; 2) a non-specific masking effect on low frequency noise on high frequency information; and 3) language specific linguistic and phonetic factors.

Danaher & Pickett (1974) have shown that the masking effect of  $F_1$  on  $F_2$  may be of both a forward and backward type and it is even possible in monaural hearing, but found no simple correlation between the extent of masking and the configuration of the pure tone audiogram. Although they measured syllable recognition rather than recognition of speech sound information, their results parallel the present ones in other respects as far as discrimination is concerned.

The recognition errors found in simulation tests concerning various types of hearing loss (Kiukaanniemi & Määttä 1979) suggest that the poor discrimination found in group C is clearly derived from two types of error: (a) the ratio between errors in vowels and errors in consonants increases markedly as the sound pressure level increases above 90 dB re  $2 \times 10^{-5} \text{ N/m}^2$ ; (b) Errors in the place of articulation remain at a relatively high level for practically all place categories at 90 dB re  $2 \times 10^{-5} \text{ N/m}^2$ . On the other hand, errors in the manner of articulation reach a lower error level with a steep decrease in errors for obstruents, i.e. consonants with vowel-like formant structure show a rising type of identification score. This fact argues in favour of the significance of the masking effect of  $F_1$  on  $F_2$  discrimination in group C.

On the other hand, the masking effect of  $F_1$  on  $F_2$  is particularly significant in languages like Finnish which are rich in vowels. In an analysis using Finnish and English speakers and phonetically balanced word material (Kiukaanniemi 1979b) the speech power of the Finnish speaker was shown to be centred significantly more on the low frequencies



Fig. 1 The macula of the saccule following ultrasonic irradiation towards the vestibule with 100 milliwatts (H&E, 25X). *es* Endolymphatic space; *n*, neuroepithelium over otolithic membrane.

1.5% veronal buffered osmium tetroxide (Brennberg et al 1965). The results were compared with that occurring after a 4-week recovery period assessed from conventional histological methods involving Heldenhaus's aluna fixation, nitrocellulose embedding, serial sectioning and staining with haematoxylin and eosin.

## RESULTS OF VESTIBULAR IRRADIATION

Vestibular disturbances observed immediately after recovery from the anaesthesia included paralytic nystagmus accompanied by a head-bobbing motion and loss of balance control with the cat falling onto its irradiated side. The severity of these effects was dependent on the intensity and duration of irradiation with the

strongest reaction following application of 100 milliwatts for 20 min. The direction of irradiation was found to be critical and was dependent on ultrasound being aimed towards the position of the ampulla of the superior semicircular canal. In most cases completely normal locomotory function was regained within ten days following the irradiation. These symptoms of balance dysfunction indicated vestibular irritation and animals not displaying this response were assumed to be incorrectly irradiated and were excluded from subsequent histological examination.

Four weeks after vestibular irradiation histological changes were observed within the vestibular neuroepithelium. Due to the variability of cellular changes it was impossible to relate the extent of damage to the ultrasound dosage and the following changes were observed following 100 milliwatts irradiation. In the saccule the otolithic membrane was often lifted away from the neuroepithelium (Fig. 1) causing damage to the hair cell cilia. The sensory epithelium lacked clarity of cellular detail and intercellular spaces occurred in this layer and in the underlying stroma. The basement membrane separating the epithelial layer from the stroma was not clearly differentiated. The otolithic membrane in the utricular macula was more resistant to damage but cellular disruption and cilia loss within the neuroepithelium was widespread. The cell walls were destroyed and the nuclei of the supporting cells and sensory cells clumped together into thin strands resulting in disorganisation of the normal stratified appearance of this epithelial layer (Fig. 2). Cellular detail in the underlying matrix was somewhat obscure.

Degenerative changes occurred to a lesser degree within the cristae in the ampullae of the semicircular canals. Deformities of the cupula commonly occurred in the superior ampulla, together with loss of cellular detail in the neuroepithelium. The extent of these changes was variable but there was a tendency for the utricular side of the crista to be more severely damaged than the semicircular canal side (Fig.

## THE EFFECT OF ULTRASONIC IRRADIATION ON THE STRUCTURAL INTEGRITY OF THE INNER EAR LABYRINTH

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*From the Ultrasonics Institute Sydney Australia*

(Received April 26 1979)

**Abstract** The histological appearance of the inner ear labyrinth was examined following ultrasonic irradiation of the vestibule and the cochlea in cats and guinea pigs. Directing ultrasound through the round window towards the ampulla of the superior semicircular canal produced severe balance dysfunction together with histological damage throughout the vestibular labyrinth. Cochlear damage was restricted to a small region of the basilar membrane proximal to the round window. When ultrasonic energy was directed into the cochlea cellular disruption extended over an area of at least two cochlear turns and included changes in the stria vascularis as well as to hair cells. The outer hair cells were found to be more sensitive than the inner hair cells to the damaging effects of ultrasound.

The alleviation of the vertiginous symptoms of Meniere's disease by the application of ultrasound into the diseased vestibule is an established practice (Arsilan 1964; James 1963; Sjöberg et al 1963; Kossoff et al 1967) developed a technique which avoids the risk of facial nerve trauma and hearing impairment by irradiating the vestibule through the round window. The success of this treatment has been confirmed from examination of patient case histories over a four year post-operative period (Barnett & Kossoff 1977). Although there have been several studies on the effects of ultrasound in the inner ear (Brain et al 1960; James et al 1964; Giancarlo et al 1965; Stahle & Sugar 1973; Lundquist et al 1978) the mechanism involved in alleviating the symptoms remains unknown. The present study was undertaken to assess the extent of pathological changes in the vestibular and auditory labyrinth following irradiation through the round window and to determine the optimal dosage for vestibular damage.

### MATERIALS AND METHODS

The round window ultrasonic applicator designed and constructed at the Ultrasonics Institute consists of a flat 3.5 MHz transducer 1.2 mm in diameter which is mounted in a cylindrical holder. Ultrasonic energy propagates in a beam diameter of 1.2 mm for a distance of 6 mm and then diverges to reach a diameter of 2 mm at a distance of 10 mm from the transducer. Irradiation was applied at average intensities of from 5 W/cm<sup>2</sup> to 10 W/cm<sup>2</sup> for a duration of 20 minutes during which the transducer was fixed in position with a micromanipulator. These intensities correspond to 50 milliwatts and 100 milliwatts respectively of acoustic power measured by the radiation pressure technique. Coupling was achieved with the irradiation site and the ultrasound transducer submerged in distilled water. Following surgical exposure of both tympanic bullae the right inner ear was irradiated while the left ear was sham-irradiated and served as a control.

In the first part of the investigation the vestibule of six cats was irradiated through the round window and the effect on cell structure was studied in the cochlea and vestibule. In a further series of experiments with six cats and six guinea pigs ultrasound was directed along the longitudinal cochlear axis from either the round window or the helicotrema. Immediate structural changes were determined by examination of surface preparations which involved the removal of the otic capsules under deep anaesthesia and fixation for 90 min with

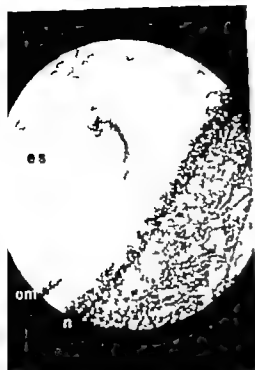


Fig. 1. The macula of the saccule following ultrasonic irradiation towards the vestibule with 100 mw/hrs (H&E, 250 $\times$ ). *es*, Endolymphatic space; *n*, neuroepithelium; *om*, otolithic membrane.

1.5% veronal buffered osmium tetroxide (Bredberg et al. 1965). The results were compared with that occurring after a 4-week recovery period, assessed from conventional histological methods involving Hendenham's soda fixation, nitrocellulose embedding, serial sectioning, and staining with haematoxylin and eosin.

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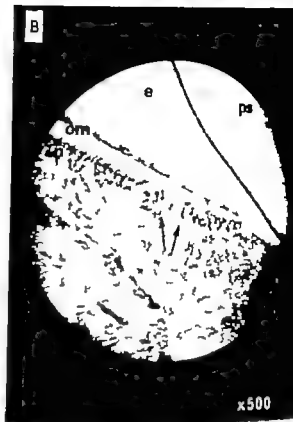


Fig 2 (A) The utricular macula from a control cat ear showing the normal pseudostratified neuroepithelium with sensory cilia projecting into the otolithic membrane (H&E  $\times 650$ ) (B) The utricular macula showing vacuo-

lation clumping of the sensory cells into thin strands and total loss of their cilia following vestibular irradiation at 100 milliwatts (H&E,  $\times 500$ ) c Cilia, cr connective tissue stroma p perilymphatic space tb temporal bone.

3) The crista of the lateral semicircular canal was rarely affected while the posterior crista and cupula appeared normal in every animal following irradiation

In the cochlea the cellular components of the organ of Corti were unchanged except for a restricted region proximal to the round window where cytoplasmic vacuolation in the inner and outer sulcus cells and some loss of outer hair cells occurred (Fig 4) In addition distortion of Reissner's membrane was observed which may possibly result in interference with the motion mechanics of the cochlear partition

#### RESULTS OF COCHLEAR IRRADIATION

The immediate effects of irradiating the cochlea through the round window were observed

during examination of surface preparations and consisted mainly of disorganisation of the outer hair cells throughout the basal turn and occasional cell losses in the second turn (Table 1) A dosage related effect was seen where 50 milliwatts produced disarray of hair cell cilia restricted to the first cochlear turn while 100 milliwatts resulted in cellular loss and stunted hair cell cilia in the first two turns (Fig 5) The supporting cells in the organ of Corti remained unchanged at the maximum irradiation dosage When examining the cochlea four weeks post-irradiation hair cell damage was more extensive and additional cellular changes occurred throughout the first two turns These included destruction of Deiter's cells and disorganisation of Claudius cells (Fig 6) Also the secretory epithelium on the endolymphatic surface of the stria vascularis showed cellular disorganisation in the basal turn and vacuo-

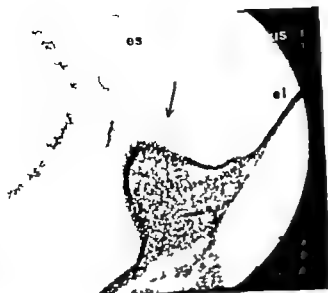


Fig 3 Histological appearance of the crista in the superior semicircular canal ampulla following irradiation into the vestibule in cats. Deterioration of the cupula and cuticular surface of the neuroepithelium was typically most pronounced on the utricular side of the crista (H&E, 500). Cupula, *el* endothelial lining of membranous labyrinth, *ms* semicircular canal side of crista, *es* utricular side of crista.

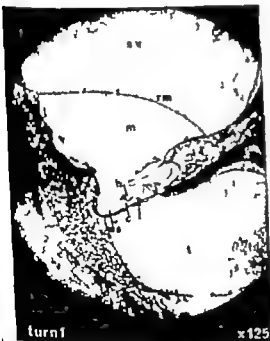


Fig 4 Vestibular irradiation with 100 milliwatts produced changes in the restricted region of the basilar membrane proximal to the round window including loss of outer hair cells (arrow), loss of nuclei from Claudius and outer sulcus cells, and the accumulation of cell debris on the endolymphatic surface of the stria vascularis (H&E, 125).

lation within the second turn. Occasionally cellular changes were found to extend into the third turn but these were restricted to vacuolation in and around the hair cells and inner sulcus cells. The endolymph contained cellular debris proximal to Claudius cells suggesting a change in permeability.

The immediate effect of irradiating the cochlear apex with 50 milliwatts was a disruption of the outer hair cells in the fourth cochlear turn. Increasing the dosage to 100 milliwatts resulted in distortion of the outer hair cells and loss of their cilia from the apical to the second turn. Losses of inner hair cells were mostly restricted to part of the apical turn (Fig. 7) and only occurred following irradiation with the higher dosage. The inner hair cells were found to be more resistant to ultrasonic damage as their cilium loss was restricted to only the apical turn compared to three cochlear turns in the outer hair cells (Table 1). Although the outer hair cells were the most sensitive of the sensory cells the degree of damage was too inconsistent to indicate preferential sensitivity of any particular row of hair cells.

Table I Surface preparation results following cochlear irradiation with 100 milliwatts for 20 min applied from the round window and the cochlear apex

| Cochlear turn | Row of hair cells | Condition of hair cells  |   |
|---------------|-------------------|--|---|
|               |                   | Round window irradiation   | Apical irradiation  |
| I             | Outer             | Mostly indistinguishable with occasional total hair cell loss but complete loss of cilia | Completely normal with typical W-arrangement of cilia   |
|               | Inner             | Normal except for disarranged cilia and rare cell loss                                   | Undamaged   |
| II            | Outer             | Cells intact but cilia disarranged and stunted with occasional losses                    | No cell losses but irregularities evident in cell outline<br>Disorientation of cilia throughout                               |
|               | Inner             | All cells unaffected with a normal continuous line of stereocilia                        | Undamaged   |
| III           | Outer             | All normal with undamaged cilia  | Where present the cell walls have an irregular outline. Cilia mostly absent and where present show gross deformity in pattern |
|               | Inner             | Normal with intact cilia   | Irregularities in the row of cilia with occasional break in continuity but cells are intact                                   |
| IV            | Outer             | Hair cells and cilia intact  | Cells and cilia completely disorganised   |
|               | Inner             | Hair cells and cilia intact  | Almost total destruction of cilia and occasional cell losses  |

## DISCUSSION

Vestibular irradiation resulted in pathological changes in the neuroepithelium and cupulae which would have a significant effect on the functional state of these organs. There was no evidence of fistulas or pores in the membranous labyrinth suggesting that any effects on membrane permeability would be of a subtle nature such as by deprivation of energy supply to active transport system. The most severe effect on vestibular function was exhibited as a unilateral loss of balance control and was only achieved when ultrasound was directed towards the position of the ampulla of the superior semicircular canal. This implies that during the treatment of Meniere's disease

vestibular ablation would be most effectively achieved by ensuring irradiation in this direction. The mechanism involved in bringing about the functional changes seems to be a destructive effect occurring specifically at the neuroepithelium rather than permeability changes in the endothelium which could be induced by applying ultrasound anywhere within the vestibule.

The dosage of ultrasound applied to the vestibule exceeded the damage threshold of neuroepithelial tissue although a relationship between the degree of damage and dosage level could not be determined. In general it was found that the dosage of 80 milliwatts applied for 20 min which is typically used in the

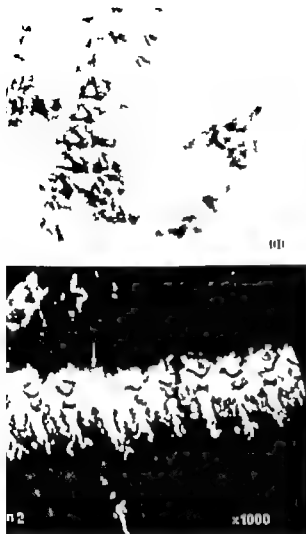


Fig. 5 (A) A surface preparation of guinea pig cochlea showing the normal regular pattern of the outer hair cell cilia (Phase contrast,  $\times 1000$ ). (B) Cochlear irradiation with 100 milliwatts resulted in stunting and distortion of the cilia, and some hair cell losses in the inner most row of the outer hair cells (P.C.  $\times 1000$ ). *lhc*: inner hair cells, *ohc*: outer hair cells; *p*: pillar cells.

d window ultrasonic treatment of Me  
s disease was sufficient to initiate patho-  
logical change

Following vestibular irradiation changes in  
structure were observed in a restricted re-  
gion of the basilar membrane proximal to the  
window. The resulting impairment of  
hearing function would involve the response  
to high frequencies above the range of what is  
considered to be practical hearing (Von  
Békésy 1960; Stevens et al. 1935). The same  
sound irradiation regime has produced no

deleterious change in the cochlear micro-  
phonic response to stimulus frequencies up to  
8 kHz (Barnett 1979).

The results of cochlear irradiation demon-  
strated progressive cellular degeneration  
whereby the extent of immediate structural  
changes increased after a post irradiation  
period of latency during which cellular ne-  
crosis became histologically discernible. The  
immediate histological changes seem to be  
due to ultrasonically induced cellular agitation  
rather than a thermal mechanism as they have



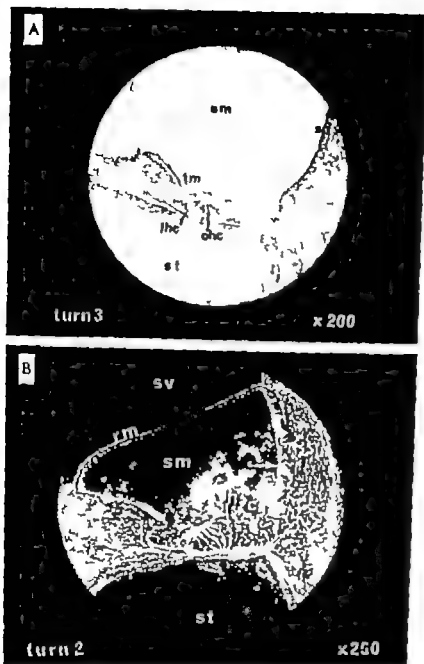


Fig 6 (A) A phase contrast photomicrograph showing the normal cytoarchitecture within the cochlear duct in a control guinea pig ( $\times 200$ ). (B) Cochlear irradiation with 100 milliwatts applied through the round window resulted in cellular disorganization within the organ of Corti and vacuolation in the stria vascularis in the first and second cochlear turn (H&E  $\times 250$ ). c Claudius cells, A Hensen's cells, rm Reissner's membrane, sv stria vascularis, sm, scala media, st scala tympani, sv scala vestibuli, tm tectorial membrane

not been observed when the intracochlear fluid temperature was raised by an amount equivalent to that occurring during irradiation (Barnett 1979). Although both ultrasound and increased temperature immediately impair hair cell function, the thermal mechanism differs by causing cell death without producing immediately visible alterations in cell structure. The delayed appearance of such changes contribute towards the observed progressive histological degeneration. The end organs of the vestibular and auditory labyrinth are similar in

their neuroepithelial composition and relation to labyrinthine fluids and as similar changes in cell structure have been observed in both regions the mechanism involved may be the same. During the ultrasonic treatment of Meniere's disease a combination of ultrasonic and thermal mechanisms result in damage at the neuroepithelium of the vestibular end organs.

Following cochlear irradiation the row of inner hair cells was damaged to a much lesser extent than the outer hair cells and a similar effect has also been reported by Criswell &

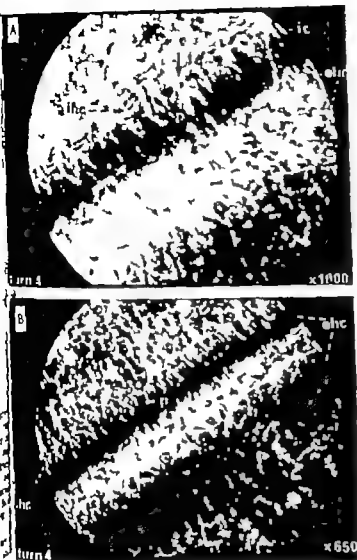


Fig 7 (A) Following irradiation with 50 milliwatts through the bone at the cochlear apex complete loss of outer hair cell cilia occurred together with occasional disruption of the regular row of stereocilia in the inner hair cells (P.C.,  $\times 1000$ ). (B) Irradiation with 100 milliwatts resulted in cellular disintegration within ohc and some loss of cilia in ihc (P.C.,  $\times 650$ ). ic: Inner hair cell cilia.

Stable (1972) when irradiating through the bone overlying the third cochlear turn in guinea pigs. It is unlikely that the resistance of the inner hair cells to ultrasound is due to their different physical location as severe damage has been observed in the nearby inner sulcus cells following inner ear irradiation in sheep (Barnett et al. 1973).

It was observed that when ultrasound affected the cochlea alterations in the stria vascularis occurred ranging from the presence of cell debris on its endolymphatic surface to cell-

ular disorganisation within its secretory epithelial layer. These changes would have a significant effect on the metabolic activity within the organ of Corti particularly as the stria is considered to be an important source of energy within the cochlea. According to Anniko et al. (1975) "a degeneration of the stria independent of reason for the degeneration is invariably followed by hair cell damage" and thus may be responsible for the progressive pathology occurring within the cochlea during the post-irradiation recovery period.

Therefore apart from the histologically evident destructive nature of ultrasonic energy biochemical changes of this type may represent a more subtle indirect effect

## ZUSAMMENFASSUNG

Vestibuläre Bestrahlung führte zur Störung der Zellen im Sacculus und im Utriculus, aber cochleäre Schädigung war minimal und beschränkte sich auf die Nähe des runden Fensters. Funktionsstörung des Gleichgewichtsmechanismus ergab es nur als die Ultraschallwellen direkt auf die Ampulla membranacea superior gerichtet wurden. Dies betonte wie wichtig es ist während Behandlung der Ménièreschen Krankheit den Ultraschallstrahl präzise zu richten, um maximale Zerstörung der vestibulären Endorgane zu erzielen und um Schädigung auf die Nähe des runden Fensters zu beschränken. Cochleäre Bestrahlung zeigte einen merklichen Unterschied an Empfindlichkeit zwischen den zwei Arten von Haarzellen im Organ des Corti, wobei die inneren Haarzellen der Ultraschallbestrahlung gegenüber weniger empfindlich waren. Das Ausmaß der Haarzellenschädigung war mit der Menge der Bestrahlung verbunden und fortschreitende Degeneration der Zellen geschah im Laufe der Erholungsperiode nach der Bestrahlung.

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## FATIGABILITY OF THE STAPEDIUS REFLEX IN INDUSTRIAL NOISE

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**Abstract** Normal hearing subjects were unilaterally exposed to 30 min of tape recorded shipyard noise (97 dB (A)), which is characterized by a variable temporal structure. The stapedius muscle activity was continuously recorded in the opposite ear as a change of the ear's acoustic impedance. The reflex latency was, in addition, assessed as stimulus-response curves obtained before and, at various times after exposure. A slight reflex fatigue was observed, together with a parallel shift of the stimulus-response curve (average 4 dB). The recovery was slow and not complete even 18 min after the end of the exposure. The individual variability was large. For 5 of the subjects the exposure was repeated at a later session and the individual degree of fatigue was found to be largely reproducible. The present results suggest that the stapedius reflex might play a role in the clinical picture of noise induced hearing loss.

Much interest has been focused on the stability and decay of the stapedius reflex response to constant pure tone stimuli and noise signals (Kato 1913, Wersäll 1958, Dallos 1964, Dyrestrand et al. 1966, Johansson et al. 1967, Tizze 1968, Coles, 1969, Kaplan et al. 1976, Lutman & Martin, 1978, Wilson et al. 1978). These studies all show very rapid adaptation of the response. It is however also known that a reflex response which has decayed will reactivate after even a very short pause (Metz, 1951, Wersäll 1958, Borg & Ödman 1979) or after a change in intensity or frequency (Gacevics & Sjöboel 1966). The sound usually resulting in damage to the inner ear—industrial noise—varies with respect to frequency and intensity. It is therefore difficult to predict from available data how the stapedius muscle acts during a workday in an industrial noise environment and thereby the possible role of

the reflex in noise induced hearing loss. The presently prevailing opinion is that the muscle rapidly adapts (e.g. Tomdorf 1976) but there are also some early observations (Lüscher 1930, Kobrak et al. 1941) which indicate that this might not be the case. Lüscher observed the stapedius tendon through a perforation in the ear drum. He described a rapid adaptation upon continuous steady sound stimulation but a remarkably great resistance to fatigue in a more varied sound environment.

The aim of the present work was to study the stapedius reflex characteristics in a realistic industrial noise environment. Normal hearing subjects have been unilaterally exposed under laboratory conditions to a 30 min sequence of noise recorded in a shipyard. The stapedius reflex was continuously recorded in the contralateral ear and stimulus-response curves at 0.5 and 2.0 kHz were obtained before and at various times after the exposure. Of particular interest were the time course of reflex recovery after the exposure and individual variability with respect to fatigue. A preliminary report has been published (Borg et al. 1979a).

## METHODS

The experiments were performed on 18 subjects aged 18 to 47 years. Their hearing thresholds were within 20 dB of ISO 1964 be

The present study was supported by grants from the Swedish Work Environmental Fund (ASF 78/111).

Therefore apart from the histologically evident destructive nature of ultrasonic energy biochemical changes of this type may represent a more subtle indirect effect

## ZUSAMMENFASSUNG

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**Procedures** After otoscopic examination and hearing threshold determination stimulus-response curves were determined for 0.5 kHz and 2.0 kHz pure tones. During the 30 min noise exposure the contralateral response was continuously recorded. The balance of the impedance bridge was continuously controlled and the subject was instructed to swallow repeatedly. After the end of the exposure the stimulus-response curves were again obtained according to the following schedule. Immediately after the end of the noise 2.0 and 0.5 kHz; after 5 min 2.0 kHz; after 10 min 2.0 and 0.5 kHz. Finally tympanometry and pure tone audiometry were repeated.

## RESULTS

The first and last minutes of the exposure noise were identical. This allowed the detection of even minor changes in reflex characteristics. Fig. 1 shows a typical example of such a sequence of recordings. The upper graph shows a recording of the noise signal wherein the high intensity periods appear diffuse. The middle graph shows the impedance change in the contralateral ear during the first minute and the lower graph shows the corresponding recording during the last minute after 30 min. It is seen that the impedance change well follows the variations in noise intensity. It is especially notable that the impact noise elicits a series of responses which summate to give a background level of muscle activity with superimposed peaks. The impedance change is altered to some extent after the 30 min exposure. Only the lower level components are depressed; stronger sounds still give maximum responses.

The stimulus-response curves obtained at various times post-exposure were compared with the corresponding curves obtained before exposure in order to quantify the alteration of the reflex and to establish the rate of recovery. Fig. 2 shows typical stimulus-response curves before (continuous line) and less than

one minute post-exposure (broken line). The stimulus-response curve after exposure is more or less parallel although shifted to the right and the maximum is unchanged. A shift of this type is compatible with a selective suppression of the low level activity as shown in Fig. 1.

In order to quantify the changes of the reflex the shift of the stimulus-response curve (B in Fig. 2) was measured and expressed as a function of sensation level ( $A = \text{dB re reflex threshold}$  Fig. 2). The suppression of the stapedius reflex after 30 min noise exposure is described in this way in Fig. 3. Fig. 3 A shows shifts at 2.0 kHz less than one min post-exposure and Fig. 3 B shows shifts 0.5 kHz about 2 min after exposure. Each subject is shown by a thin continuous line and the mean value  $\pm s.e.$  is shown by the heavy line. It is seen that the variability is appreciable. Some subjects exhibit an improvement of response; others suffer more or less pronounced fatigue. The reflex depression was significant ( $P < 0.05$ ) only at 2.0 kHz (5 and 10 dB re reflex threshold). The average shift is largely independent of the sound level which is indicative of a parallel shift rather than a change of slope of the stimulus-response curve. In conclusion there is a small but significant reflex fatigue following an exposure to the 30 min sequence of industrial noise.

To investigate the remarkable spread of data seen in Fig. 3 five subjects were reexposed to the same noise from a few days to one year later and retested.

Fig. 4 shows pairs of curves for each subject representing alterations of reflex properties based on 2.0 kHz pure tone stimulation less than one min post-exposure. The individual mean values are indicated to the right in the graph. It is seen that one subject has consistently large shifts in one direction while another one consistently showed a potentiation of the reflex (negative values). The remaining subjects show intermediate reactions. Although no quantitative estimation of reproducibility can be made the results presented

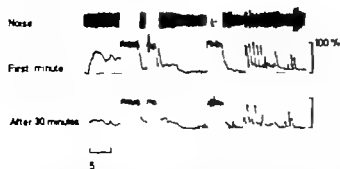


Fig 1 Response of the contralateral stapedius muscle measured as impedance change to shipyard noise. Upper graph: Exposure noise. Middle graph: Impedance change during the first minute of the exposure. Lower graph: Impedance change during the 30th min in response to an identical noise sequence. Level during this minute: 114 dB (A).

tween 0.125 and 8.0 kHz. They had no history of ear disease and their ear drums were normal upon otoscopic examination. Reproducibility was assessed by repeated measurements on 5 of the subjects.

**Recording of stapedius muscle activity.** The response of the muscle was measured as a change of the ears acoustic impedance (Lidén et al 1972). The 0.8 kHz probe signal (70 dB SPL) was electrically balanced with respect to phase and amplitude. The muscle contraction therefore can be measured as the length of the complex impedance change vector. The stimulus response curve of the reflex was obtained by presenting 1 sec bursts of 0.5 or 2.0 kHz pure tones in the contralateral ear. The sound level was raised in 5 dB-steps from 70 dB HL to 120 dB HL and thereafter lowered from 120 to 70 dB HL. The middle ear pressure was determined with tympanometry and appropriately adjusted. The subjects were instructed to swallow repeatedly during and after the noise exposure to avoid pressure abnormalities.

**Noise exposure.** The ear contralateral to the probe ear was exposed to 30 min of industrial noise presented from a tape recorder (Revox A77) via an audiometer (Madsen OB 70) and an earphone (TDH39 MX41AR). In order to minimize interference with the probe tone, the exposure noise was fed through a notch filter with a center frequency at 0.8 kHz with a

reject band from 0.7–0.96 kHz (General Radio 1952, 36 dB per octave).

The tape recording of the noise was done earlier in the welding shop at the shipyard (Nilsson et al 1977) and is typical for the noise exposure of welders and platers. This type of noise contains impulses from sledge hammers and also high sound levels from pneumatic hammers and striking not tighteners. These impulses are superimposed on a fluctuating background noise level. The 30 min portion of the recording used in this test had an equivalent continuous noise level of 97 dB (A).

The first minute of the recording which contained tightener noise and impact noise was copied and added to the end of the recording in order to determine fatigue in detail.  $L_{eq}$  during this minute was 114 dB (A). The equivalent continuous level of the band-reject filtered noise was measured by a 1/1 microphone B&K 4145, 6 cc coupler and a noise average meter which integrates the noise level according to ISO standard ( $q$  factor=3 dB).

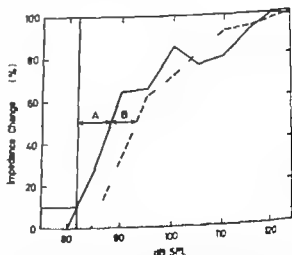


Fig 2 Effect of 30 min industrial noise 97 dB (A) on the stimulus-response curve of the stapedius reflex at 0.8 kHz. Impedance change shown in percentage of maximum obtainable change as a function of sound level. The continuous line is obtained before exposure and the broken line less than 1 min post-exposure. Threshold is defined as 10% of maximum impedance change and A denotes dB above reflex threshold. B is the increase of stimulus level necessary to obtain the same impedance change pre- and post-exposure.  $e$  the amount of shift.

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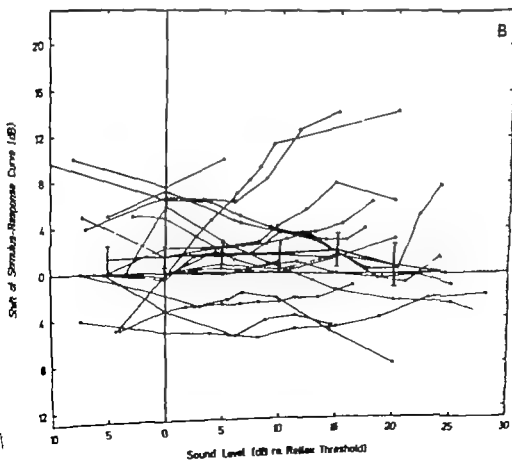
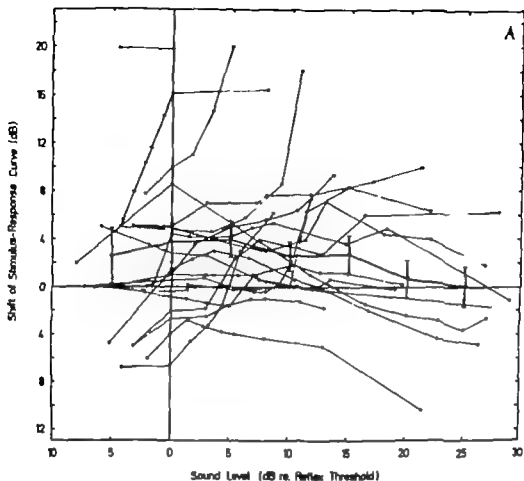
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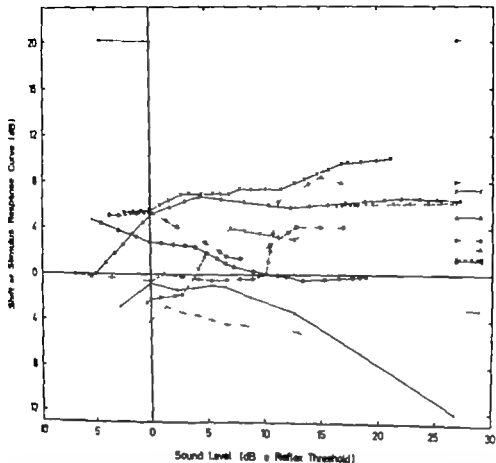


Fig. 4. Reproducibility of stapedius reflex fatigue after 30 min noise exposure (97 dB (A)) obtained in 2 experimental sessions in 5 subjects. Continuous line: first session; broken line: second session. The abscissa shows dB re individual reflex threshold (A of Fig. 2). The ordinate shows the

change in sound level after noise exposure necessary to produce the same impedance change pre- and post-exposure (B in Fig. 2). 0 kHz stimulation, less than 1 min post-exposure. Mean values for each measurement is shown to the right.

in Fig. 3 and 4 seem to reflect a true variability between individuals.

The presentation thus far has been restricted to the reflex suppression immediately post exposure. Although the mean suppression is

Fig. 5. Shift of stapedius reflex stimulus-response curves after 30 min noise exposure (97 dB (A)) measured as the increase of sound intensity necessary to produce equal impedance changes pre- and post-exposure. The shift (B of Fig. 2) is expressed as function of sensation level (A of Fig. 2) re individual reflex threshold (A of Fig. 2). Positive values indicate fatigue and negative values potentiation. A: test frequency 2.0 kHz; B: test frequency 0.5 kHz. Mean values and standard error are indicated by heavy lines.

small, it is possible to see a recovery during the time of observation (10 min). The mean and standard error of the shift of the stimulus-response curves after 30 sec, 5 min and 10 min tested at 2.0 kHz is illustrated in Fig. 5. The filled circles represent all 13 subjects and the open circles represent the four subjects with an initial suppression more than 7 dB. There is a recovery which is not complete after 10 min. The time course can be well approximated both with a straight line (broken lines) and with an exponential function (continuous lines).

In conclusion the stapedius reflex is active

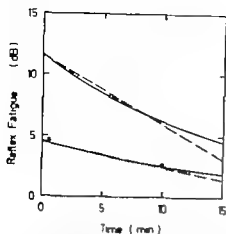


Fig. 5 Fatigue of the stapedius reflex obtained as shifts of stimulus-response curves at 2.0 kHz at various times post-exposure. Mean values are based on individual shift curves for all 13 subjects (filled symbols) ( $y = -0.21x + 4.5$ ,  $y = 4.5e^{-0.04x}$ ) and for those 4 subjects with the largest suppression of the reflex (open symbols) ( $y = -34.33x + 11.6$ ,  $y = 11.47e^{-0.04x}$ ).

throughout a 30 min exposure to industrial noise. Generally the response to low level sounds adapts slightly and the individual variability is striking. Recovery can be approximated by an exponentially decaying function or straight line with a negative slope.

## DISCUSSION

The stapedius reflex showed only slight decay after a 30 min exposure to industrial noise; recovery, however, was slow. This resistance of the reflex to fatigue found in the present study contradicts the rapid decay during constant pure tone stimulation but confirms earlier observations by Lüscher (1950) and Kobrak et al (1941). A detailed investigation of the decay of the stapedius reflex during stimulation with synthetic complex and time varying sound has recently been published (Luthman & Martin 1978). Very little reflex decay was observed for stimuli of up to 5 min duration (repetitive 50 ms noise bursts with 50 ms intervals). The slow recovery found in the present study is in contrast to the very rapid recovery from decay to short high frequency continuous tones (Borg & Ödman 1979). This does not necessarily indicate, however, that

two separate mechanisms operate. In single unit recordings from auditory nerve fibres, Yound & Sachs (1973) found a decay to continuous pure tone stimulation which recovered more slowly with longer exposures and higher intensities. Even though decay to a short sound almost exclusively is a matter of the afferent part of the reflex arc, a component of muscular fatigue cannot be excluded from the present result. In a parallel field study (Borg et al 1979b) with bilateral reflex recordings the muscular function was found to be unaffected after 5–7 h of exposure. In addition the slight difference between fatigue at 0.5 kHz and 2.0 kHz favours this interpretation.

Although the present findings do not prove that the muscle is active throughout a noisy workday, the observed fatigue-resistant supports such an assumption. The inter-individual variability observed was striking and some of the subjects showed more fatigue than the majority. This suggests that the stapedius reflex might be one source for individual variability in susceptibility to noise induced hearing loss.

## ZUSAMMENFASSUNG

Achtzehn normalhörende Personen wurden auf einem Ohr 30 min lang mit auf Band aufgenommenem Schiffsbauhub (Äquivalenzniveau  $L_{eq} = 97$  dB (A)) der in seiner Struktur zeitlich variabel ist, beschallt. Während der Untersuchungen wurde im Gegenzug die Aktivität des Stapediusmuskels kontinuierlich als relative Impedanzänderung aufgezeichnet. Des weiteren wurden auch die Reflexfunktion als Reflex-Response-Kurve vor und zu verschiedenen Zeitintervallen nach der Beschallung aufgenommen. Als Ergebnis wurde eine leichte Abnahme des Reflexes und eine gleichzeitige Parallelverschiebung der Reflex-Response-Kurve um in Durchschnitt 4 dB beobachtet. Der aus der Reflex-Response-Kurve ersichtliche Rückgang der Reflexabnahme nach der Beschallung war langsam und noch nach 10 min nach dem Ende der Beschallung nicht vollständig. Allgemein zeigten die Messungen eine erhebliche interindividuelle Variabilität. Bei einer Wiederholung der Lärmbelastung an fünf der Versuchspersonen in einer späteren Sitzung zeigte sich der individuelle Grad der Reflexabnahme weitgehend reproduzierbar. Abschließend ist zu bemerken, daß der vorliegende Befund der weitgehenden Ermüdungsresistenz des Stapediusreflexes während der Beschallung vermuten läßt, daß er in dem klinischen Bild der durch Lärm verursachten Hörschädigung eine Rolle spielt.

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# ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL CHANGES IN THE GUINEA PIG COCHLEA FOLLOWING MECHANICAL TRAUMA TO THE ORGAN OF CORTI

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**Abstract** Small discrete lesions were produced in the organ of Corti of the guinea pig cochlea using fine probes to produce direct mechanical insult. The electrophysiological state of the cochlea was assessed using M electrocochleography and loss of receptor cells determined by scanning electron microscopy. Principal findings were: 1) Excellent agreement between the location of hair cell losses and the frequency of maximum sensitivity change in the N audiogram. 2) The spatial extent of the mechanically induced lesion appears to be more important than the total number of hair cells lost, in determining the magnitude of N sensitivity loss. 3) Hair cell losses extending over only 72  $\mu$ m could be detected as significant changes in N sensitivity. These results further emphasize the accuracy and usefulness of the N electrocochleogram for assessing the functional status of the cochlea. 4) Lesions involving only outer hair cell loss also produced marked elevations of N threshold.

One traditional approach to the general problem of structure-function interrelationships in the cochlea has been to produce lesions in the organ of Corti and study the subsequent magnitude and extent of the auditory deficit. The results of many of these studies have been paradoxical and anomalous. For example hearing losses have been reported accompanied by no apparent damage to receptor cells (Hunter Duvar & Elliott 1972 1973 Dolan et al 1975). Conversely relatively large lesions produce no corresponding hearing loss (Schuknecht 1953 Stebbins et al 1973 Bredberg & Hunter Duvar 1975).

In a recent review Bredberg & Hunter Duvar (1975) pointed out many of the methodological and technical problems which could contribute to such paradoxical findings. Limitations exist in both the methods used to pro-

duce and evaluate the morphology of lesions and in the techniques chosen to assess the functional status of the cochlea. In particular the use of intense acoustic stimulation to produce receptor cell losses gives generalized effects which render interpretation of changes in cochlear output difficult. Assessment of lesions using light microscopy has the limitation of not revealing fine ultrastructural changes which may be functionally important while serial sectioning can result in spatially restricted hair cell losses not being detected. The measurement of behavioural audiograms in experimental animals is also tedious and has inherent inaccuracies.

Our approach has been firstly to produce spatially restricted lesions by direct mechanical insult to the organ of Corti in a reproducible location on the cochlea partition. Secondly the anatomical assessment of these lesions has been performed using high resolution scanning electron microscopy. Thirdly we used the thresholds of the gross cochlear nerve action potential in response to tone bursts of varying frequency (N<sub>1</sub> electrocochleogram) to investigate the functional status of the lesioned cochleas. There is accumulating evidence that the electrocochleogram is an accurate and sensitive probe of cochlea function (Ruben et al 1976 Johnstone et al 1978 Dallos et al 1978). The present study provides additional support for this notion.

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## MATERIALS AND METHODS

Twenty-one healthy young pigmented albino pigs (140–400 g) with normal plasma reflex were used in this study. They were anaesthetized intraperitoneally with 50 mg/kg of pentothal® (sodium pentothal) mounted on rigid hollow ear bars and the entrance to the left bulla gained with a dorsolateral approach under sterile conditions.

A sterile silver wire electrode was placed on the bony shelf near the round window to record the gross neural response (N) to gated tone bursts (rise time 1 msec, 50 msec duration, repetition rate 4 per second). A calibrated closed sound delivery system was used identical with that described by Wilson & Johnstone (1975) the stimuli being delivered to the left tympanic membrane through the ear bar inserted in the external auditory meatus. N responses were amplified by a Grass P15 AC preamplifier (bandwidth 100 Hz–3 kHz) and displayed on a cathode ray oscilloscope (Tektronix 502). An electrocochleogram was obtained by measuring the N threshold (estimated by the visual detection method) for tone frequencies ranging from 2–30 kHz. These thresholds were reproducible to within  $\pm 3$  dB (Goharzadeh et al. 1978).

In order to produce a lesion in the organ of Corti the mucoperiosteum overlying the basal turn of the scala tympani wall was removed and a small hole (80  $\mu$ m) hand-drilled in the outer bone with a triangular needle taking care to avoid the spiral ligament. N thresholds were again measured to check for any damage caused by this initial opening. A glass pipette electrode (20  $\mu$ m tip diameter) filled with 150 mM KCl was then introduced via the scala tympani opening and passed through the basilar membrane and in some cases Reissner's membrane. The position of the electrode tip was followed by monitoring the d.c. potential difference between the electrode and a neck reference (Keithley 610C Electrometer). The first large negative potential (–60 to –80 mV) indicated entry into the organ of Corti. Ap-

pearance of the large +80 to +100 mV endocochlear potential signalled entry into scala media (Johnstone & Sellick 1972). The electrode was then withdrawn and a post-lesion electrocochleogram taken to measure the effect of the mechanically induced lesion on the N<sub>1</sub> thresholds. Small squares of Gelfilm were placed over the hole in the cochlea wall and the opening into the bulla. The skin overlying the bulla was sutured and the animal allowed to recover for periods ranging from 4 to 43 days. No antibiotics were administered.

For the final experiments at the end of the recovery period the animals were anaesthetized intra-peritoneally with Nembutal® (sodium pentobarbitone 60 mg/kg) and paralysed with Alloferm® (Roche) (4 mg/kg). The trachea was cannulated, the animal placed on ear bars and artificially respired with carbogen (5% CO<sub>2</sub>, 95% O<sub>2</sub>).

The bulla was opened via the original surgical entrance and the middle ear examined for signs of otitis media or infection. Two of the animals had middle ear infections and were excluded from this study. In all animals the hole in the cochlea wall had been sealed by sclerotic bone growth.

Post recovery N thresholds were determined using techniques identical with those described above for comparison with the initial and post-lesion N thresholds. Further experimentation consisted of single neurone recordings from the spiral ganglion according to the method of Robertson & Manley (1974). These results are to be reported in another communication.

Both left and right cochleas were then prepared for histological examination. The animal was decapitated at the end of the acute experiment and both temporal bones removed rapidly from the skull. The stapes were excised, the round window membranes ruptured and the perilymphatic spaces gently perfused with 3% glutaraldehyde in 0.1 M phosphate buffer within 2 minutes of sacrifice. After 1½ hours in glutaraldehyde the cochleas were rinsed in saline and post-fixed in 1% osmium tetroxide.

in 0.1 M phosphate buffer for a further 1½ hours

The specimens were then dehydrated to 70% alcohol and dissected to expose the basal coil of the cochlea

The cochlear wall of scala vestibuli was thinned down using a Kerr Electrotorque drill with a 0.9 mm diamond burr to the level of the basilar membrane and detached. The stria vascularis was then carefully picked away to expose the organ of Corti. Both Reissner's membrane and tectorial membrane were removed.

Each cochlea was photographed in the dissected state with a Wild M7A stereomicroscope in order to locate the position of any visible lesion (evidenced usually by loss of fibres) in the basal coil. Using an eyepiece micrometer (Olympus OSM) measurements were taken to locate the position of the spiral ganglion recording sites in relation to the lesion.

After all dissection and measurements had been completed the ligand-linking osmium tetroxide-thiocarbohydrazide method (Malick & Wilson 1975) was used to metal impregnate the specimens which were critical point dried with CO<sub>2</sub> in a Polaron E 3000 critical point drying unit (Bredberg 1977) mounted on stubs and examined in a Philips PSEM500 scanning electron microscope (SEM) at magnifications ranging from  $\times 10$  to  $\times 7000$ .

On completion of SEM examination of the organ of Corti the cochlea was further dissected to expose the hook region. This enables absolute distance measurements from the end of the basilar membrane to be made. The lesioned area was identified by landmarks noted during scanning microscopy.

## RESULTS

### *Pre- and post lesion electrocochleograms*

The general shape of the normal N electrocochleogram is shown in Fig. 1. The electrocochleogram consists of a low frequency por-

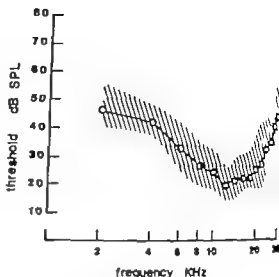


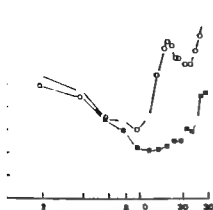
Fig. 1. Mean and range for the normal electrocochleogram of the guinea pig (a random sample of 10 animals from this study) as determined by N<sub>1</sub> threshold sensitivity using the visual detection method.

tion with a mean slope of about 20 dB/octave with sensitivity increasing to a plateau region between 12 and 22 kHz. Above 22 kHz, sensitivity falls off at around 40 dB/octave. The most sensitive N<sub>1</sub> threshold is at 12 kHz (mean 20.6 S.D. 1.4 dB SPL). These general features agree well with previous descriptions from our laboratory (Johnstone *et al.* 1978).

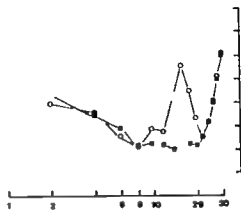
Opening of the scala tympani in preparation for basilar membrane lesioning caused bearing losses in nine of 19 ears. The loss in sensitivity was restricted to a narrow range of frequencies and is termed a notch. The maximum loss occurred in the range 16–18 kHz (mean 17.2 S.D. 1.0). The mean magnitude of the loss was 34.7 dB (range 15–79 dB S.D. 11.1 dB). Two animals (GP 59–65 (Fig. 2)) showed in addition to the notch a high frequency loss of 26–37 dB at frequencies above 22 kHz. In 4 animals no puncture of the basilar membrane was made, whereas in 5 a puncture was made despite the loss on opening the scala tympani.

Two sham operations were performed in which successful loss free openings were made in the wall of scala tympani using techniques identical with the above. The scala tympani hole was sealed without lesioning the organ of Corti.

GP 65



GP 71



frequency kHz

Types of hearing loss (■ pre-lesion, ○ post-lesion) when the organ of Corti is mechanically injured. The type of loss shown by GP71 is termed

notch, while GP65 shows in addition a loss in N sensitivity at the higher frequencies.

rupturing of the basilar membrane and in cases Remaker's membrane was carried 13 animals. Twelve of these (one was completely measured on this occasion) showed an immediate loss of sensitivity in form of a high frequency notch. The total loss of the notch immediately post-lesion (within 5 min) occurred at frequencies ranging from 13 to 20 kHz with a mean of 16 kHz (S.D. 2.0). The magnitude of the loss varied from 9 to 56 dB with a mean of 28.5 dB (S.D. 15.6).

No significant changes from pre-lesion thresholds up to 8 kHz were evident in the post-lesion electrocochleogram for all animals. In eight ears where no loss occurred on opening the scala tympani the mean loss after rupturing was 1.6 dB (S.D. 13.1 range 5 dB) and occurred at 13–20 kHz (mean 16 kHz, S.D. 2.5).

In four ears showing a loss on opening scala tympani the additional effect of puncturing the basilar membrane was studied. Mean loss before puncture was 35.0 dB (S.D. 10.4) at 16 kHz (S.D. 0.5) and after puncture 44.1 dB (S.D. 10.7) at the same frequencies. Thus the additional effect of puncturing the basilar

membrane on the hearing loss was 7.1 dB (S.D. 2.7).

#### Post recovery Electrocochleograms

Recovery period varied from 4 to 43 days. Post-recovery electrocochleograms in animals showing initial hearing loss showed improvement in post-lesion threshold in all but 2 of the animals. Three of the animals had a recovery period of 4–8 days, 8 had recovery periods of 17–21 days and 9 had recovery periods of 27–43 days. No significant differences occurred in the amount of recovery between these three groups.

The 4 animals which showed a loss on opening the scala tympani and in which the basilar membrane was not punctured showed a very small recovery (mean 2.5 dB, S.D. 5.3 dB). The eight ears which were punctured and did not have a loss on opening scala tympani showed slightly more pronounced recovery (mean 7.9 dB, S.D. 12.0 dB). Three of the animals included in this group showed complete recovery (Fig. 4). The 4 animals which showed loss on opening scala tympani—and in addition the basilar membrane was punctured—



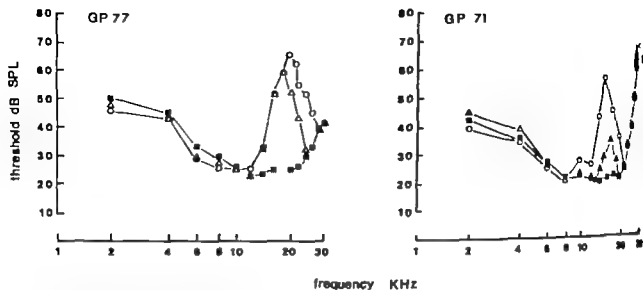


Fig 3 Normal pre-lesion electrocochleogram (●) post lesion (○) and post-recovery (Δ) for GP77 and 71. GP77 showed a loss on opening scala tympani and the organ of

Corti was not pierced. GP71 showed no loss on opening the scala tympani and the organ of Corti was pierced.

showed the greatest recovery (mean 15.8 dB S.D. 6.5 dB).

The low frequency slopes of the notch in post recovery electrocochleograms had a mean of 55.1 dB/octave (S.D. 6.8 dB) while the high frequency slopes of the notch showed a mean of 61.7 dB/octave (S.D. 9.4 dB). When compared with the post lesion electrocochleogram there was little change in the low frequency slope (52.8–55.1 dB/octave) while the high frequency slope was on average 50% steeper (43.3–61.7 dB/octave). Thus the general effect of recovery was to decrease the frequency range of the notch in the electrocochleogram and also reduce its depth. The centre frequency (CF) of the notch after recovery shifted towards a higher frequency in 6 of the animals (max 2 kHz shift). 2 animals shifted to a lower frequency (max 1 kHz shift) and the remainder showed no shift in the CF of the notch.

Of the 16 animals which showed initial hearing loss due either to opening of the scala tympani or puncturing of the basilar membrane or both, 13 showed a persistent notch with a mean of 27.9 dB (S.D. 10.9, range 12–40.5 dB). Four of these animals had additional high frequency losses beginning at 20–24 kHz and amounting to 30–46 dB at the most.

The four ears which showed loss on opening the scala tympani and were not punctured showed a mean hearing loss of 36.8 dB (S.D. 3.8). The eight ears that were punctured and had no loss on opening scala tympani showed a post recovery hearing loss averaging 13.7 dB (S.D. 14.4, range 0–40 dB). The four ears that showed loss on opening scala tympani and the basilar membrane was punctured showed post recovery hearing loss averaging 26.4 dB (S.D. 10.5, range 12.5–38 dB).

No difference in hearing loss either acute

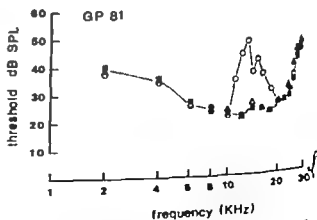


Fig 4 N threshold sensitivity curves (symbols as in Fig. 3) showing the complete recovery that occurred after the initial post-lesion hearing loss.

it-recovery was found between puncture of basilar membrane only or in addition of Renssner's membrane.

A remarkable feature of the data was the excellent reproducibility of  $N_1$  thresholds below 2. Despite the fact that up to 43 days had elapsed between pre-lesion and post recovery measurements and despite the difference in anaesthesia in the two situations pre- and post recovery low frequency

thresholds were within  $\pm 3$  dB of each other and often were identical (Figs. 3, 4, 6, 7).

An excellent correlation ( $r=0.84$ , significant at 99.9% level) was found between the peak loss in decibels of the post recovery  $N_1$  thresholds and the width in kHz of the notch in the electrocochleogram. Width was measured from the point where high and low frequency slopes intercepted the normal pre-lesion electrocochleogram. The two sham-operated gui-

*Fig. 5a. Lesioned organ of Corti showing loss of OHCs only. One OHC is in the process of degeneration (arrow) while the rest of the OHC and the IHCs appear anatomically normal.*

*Fig. 5b. High power magnification ( $\times 7000$ ) of normal OHC. Stereocilia are tightly packed and regular in their structure.*

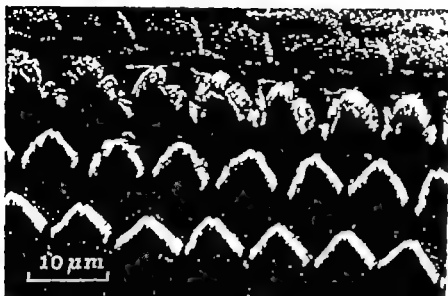


Fig. 5c. Low-power magnification ( $\times 3500$ ) of OHCs shown in areas of degeneration. The normal (QP75) showed a loss with the opening of scalary regions.

nea pigs showed no significant change in  $N_1$  thresholds between the initial and final measurement of the audiogram with recovery periods of 36 and 38 days respectively.

#### *Morphology of the Mechanically induced Lesion*

Of the 16 animals that showed a hearing loss (as estimated by the electrocochleogram) 13 also showed hair cell loss restricted in spatial

distribution and located in the basal turn of the cochlea.

The lesions were characterized by a rather sharply demarcated region within which hair cells were either missing or obviously abnormal in their surface structure. Changes in the cuticular plates or stereocilia as seen under SEM compared with hair cell structure in normal cochleas from the same weight range of animals were termed abnormal. For over-

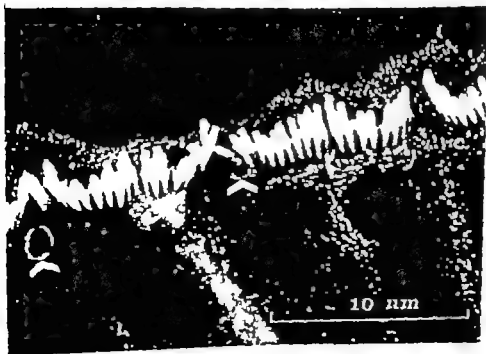


Fig. 5d. Normal inner hair cells (QP71) showing regular arrangements of stereocilia and normal distribution of microvilli. Spheroids (arrows) are probably preparation artefacts.

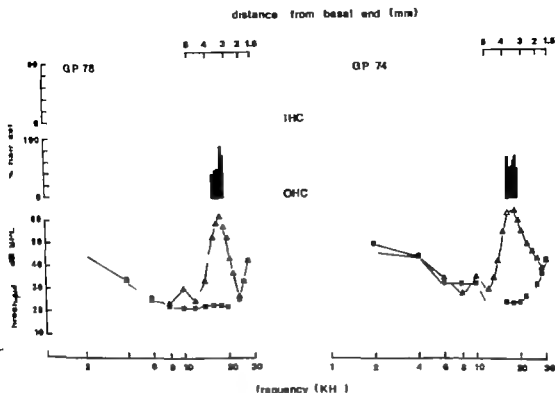


Fig. 4. Upper portion of figure shows hair cell loss (both OHC and IHC) plotted as function of distance from basal end of basilar membrane. Hair cell counts are expressed as percentage loss per 100  $\mu$ m (or groups of 10 outer hair cells). Lower portion of figure shows the N1 electro-

cochleogram ( $\Delta$ , post-recovery N1 thresholds). The relationship between the N1 thresholds and spatial location of the lesion is explained in the text. GP 74 and 78 both lost only outer hair cells and had no hearing loss on opening the scala tympani: the organ of Corti was not pierced.

venience in estimating the size of the lesion abnormal hair cells were scored as absent. Neither of the 2 sham-operated guinea pigs showed hair cell losses or structural abnormalities in the region of the cochlea in question.

The width of these lesions varied from 70  $\mu$ m to 1.1 mm with an average width of 486  $\mu$ m. In all but 4 animals both inner and outer hair cells were lost or showed severe abnormalities, but in every case more outer hair cells (OHC) were lost than inner hair cells (IHC). OHC loss generally extended both basally and apically beyond the region of IHC loss.

In the 4 animals in which a loss of N1 sensitivity occurred on opening the scala tympani, hair cell damage was restricted to the outer

hair cells. The loss of OHCs was greatest in the third row and decreased dramatically towards the first (innermost) row. All inner hair cells were present and appeared structurally normal in these animals. An example of SEM results from a lesioned cochlea is shown in Fig. 5. A complete description of the morphology of these lesions is included in a paper in preparation.

#### *Relation between Post recovery Electrocochleograms and Cochlear Morphology*

Examples of the type of correlation found between post-recovery electrocochleograms and hair cell loss are shown in Figs 6 and 7. In these figures the anatomical lesion has been plotted as a function of distance from the basal

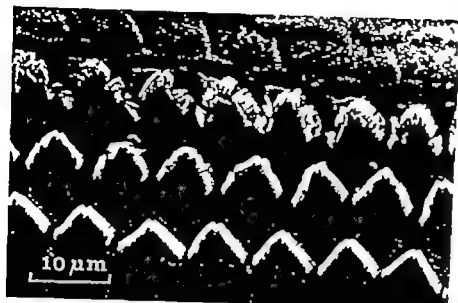


Fig. 5c. Low-power magnification ( $\times 3500$ ) of OHCs shows a range of stages of degeneration. The animal (GP75) showed a loss with the opening of scala tympani.

neal pigs showed no significant change in  $N_1$  thresholds between the initial and final measurement of the audiogram with recovery periods of 36 and 38 days respectively.

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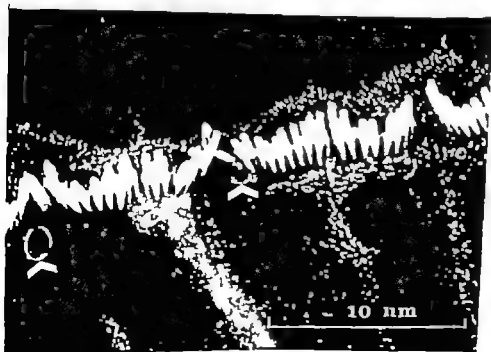


Fig. 5d. Normal inner hair cells (GP71) showing regular arrangements of stereocilia and normal distribution of microvilli. Spheroid (arrowed) are probably preparation artefacts.

mission electron microscopy (TEM) may reveal ultrastructural abnormalities within the actual cell body. No studies of this nature were carried out in this experiment.

## DISCUSSION

The sequence of events following mechanical rupture of the organ of Corti and subsequent degeneration of the receptor cells is not obscure. Introduction of a 20  $\mu\text{m}$  glass needle through the cochlear partition in this study produced loss of hair cells for distances up to 1.1 mm. One possible mechanism which may result in hair cell degeneration is a tearing of the cochlear fluids, perilymph and endolymph. Disruption of the integrity of both Reissner's membrane and the cochlear partition in other studies has produced loss of receptor cells similar to that found in this series of animals (Lawrence 1966; Duvall & Rhodes 1977; Duvall et al. 1969). In our experiments thresholds were not followed for more than 1 min after piercing the organ of Corti and consequently it was not possible to assess the full extent of the hearing loss until post-operative electrocochleograms were measured. Because the final lesion was in all cases much more extensive than the size of the objective mechanical disruption of the organ of Corti, it seems a remote cause of the final loss of the receptor cells. In any case this method is enabled spatially restricted, reproducible loss of hair cells to be correlated with the resultant deficiencies in the N1 electrocochleogram.

The use in this study of SEM rather than serial sectioning and surface preparation (Engström et al. 1966) allows for a greater degree of accuracy in assessing the morphological condition of the organ of Corti. In serial sectioning, as Bredberg & Hunter-Duvar (1975) have pointed out, every 10th section is generally stained and inspected, which means that small lesions may possibly be missed. No such inaccuracy exists in our data. Lesions as small as 3  $\mu\text{m}$  (Fig. 5a) incorporating only 8 OHCs

are clearly visible and the absence of only one hair cell is easily detectable. In addition the high resolution of SEM will reveal surface structural abnormalities which may go undetected with light microscopy. We have assumed that obvious abnormalities in cuticular plates and stereocilia imply abnormal functioning of the hair cell.

Several important points emerge from our results. Firstly the excellent correlation between location of maximum hair cell loss and frequency of maximum loss of N1 threshold sensitivity provides an additional validation of the place-frequency map of the guinea pig cochlea, even though our lesions were confined by necessity to a fairly restricted region of the basal cochlear spiral. The most complete place-frequency maps have hitherto been derived from basilar membrane mechanical measurements (Wilson & Johnstone 1975) and from spiral ganglion recordings (Robertson & Manley 1974; Johnstone 1977) both methods showing substantial agreement. From data provided by Wilson and Johnstone (1975) the equation

$$f = 45e^{-0.271x} \quad (1)$$

can be derived where  $f$  is the cut-off frequency for the basilar membrane tuning curve and  $x$  is distance from the basal end of the basilar membrane. Excellent agreement was found in our present data between the frequency of maximum loss in the N1 electrocochleogram and the location of hair cell loss when these were aligned according to the above equation. Thus eq. (1) appears to reflect accurately the spatial representation of frequency in auditory nerve fibres. The present N1 data, together with recent spiral ganglion cell recordings (Robertson et al. in preparation) from our laboratory suggest that the primary nerve fibres at a given location respond best to the frequency corresponding to the high frequency cut-off rather than the peak frequency of the basilar membrane tuning curve at that location (Wilson & Johnstone 1975). This may have im-

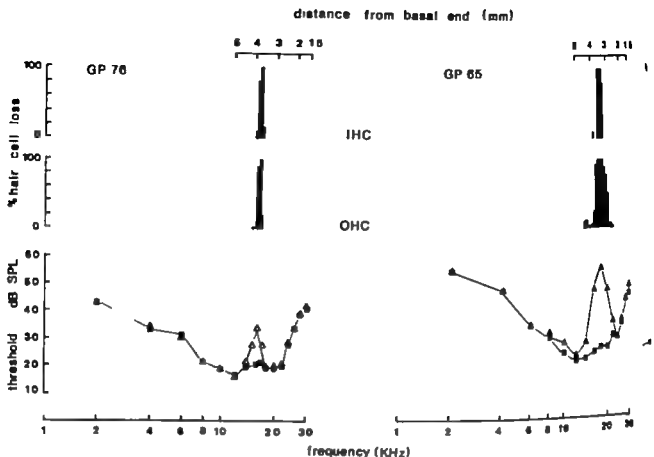


Fig. 7 Similar to Fig. 6 but GP76 and 65 both showed a mixed loss of inner and outer hair cells. No loss was incurred on opening scala tympani and organ of Corti was pierced in both cases.  $\Delta$ , Post recovery N thresholds

end of the basilar membrane and then aligned with the lower frequency scale according to the place frequency map of Wilson & Johnstone (1975) and Robertson & Manley (1974).

In all cases the entire anatomical lesion fell within the confines of the notch in the  $N_1$  electrocochleogram. All animals showed the greatest percentage hair cell loss occurring at or within 1.5 kHz of the peak of the  $N_1$  threshold loss. Fig. 7 shows 2 animals which showed mixed inner and outer hair cell lesions. Of particular interest are the results shown in Fig. 6. This illustrates 2 of the 4 animals which exhibited pure outer hair cell lesions due to scala tympani opening. In both these animals only the outermost row of hair cells was missing. Once again however the agreement between frequency of maximum  $N_1$  threshold change and the location of hair cell loss is excellent. The peak loss in decibels of  $N_1$  sensitivity

shows a much better correlation with the anatomical width of the lesion ( $r=0.75$  significant at 99% level) than with the total number of hair cells lost ( $r=0.48$  not significant). Thus GP78 (Fig. 6) in which only 139 hair cells (all outer hair cells) were lost over a distance of 700  $\mu$ m showed a peak loss at 18 kHz of 40 dB. In contrast GP72 which had 164 OHC and 39 IHCs missing but restricted to a width of 400  $\mu$ m had a peak loss of only 27 dB at 16 kHz.

In all animals the hearing loss in the electrocochleogram had a greater frequency spread than the anatomical hair cell loss. This appears to indicate that although the hair cells either side of a lesion appear to be anatomically normal functionally their thresholds are elevated. High power SEM magnifications of hair cells immediately adjacent to the lesion showed no surface abnormalities. It is possible that trans-

mission electron microscopy (TEM) may reveal ultrastructural abnormalities within the actual cell body. No studies of this nature were carried out in this experiment.

## DISCUSSION

The sequence of events following mechanical disruption of the organ of Corti and subsequent degeneration of the receptor cells is as yet obscure. Introduction of a 20  $\mu\text{m}$  glass probe through the cochlea partition in this study produced loss of hair cells for distances of up to 1.1 mm. One possible mechanism which may result in hair cell degeneration is a surge of the cochlear fluids, perilymph and endolymph. Disruption of the integrity of both Reissner's membrane and the cochlea partition in other studies has produced loss of receptor cells similar to that found in this series of animals (Lawrence 1966; Duvall & Rhodes 1967; Duvall et al. 1969). In our experiments  $N$  thresholds were not followed for more than 4 mm after piercing the organ of Corti and consequently it was not possible to assess the real extent of the hearing loss until post-recovery electrocochleograms were measured. Because the final lesion was in all cases much more extensive than the size of the probe, pure mechanical disruption of the organ of Corti seems a remote cause of the final loss of the receptor cells. In any case this method as enabled spatially restricted, reproducible loss of hair cells to be correlated with the resultant deficiencies in the  $N$  electrocochleogram.

The use in this study of SEM rather than serial sectioning and surface preparation (Engstrom et al. 1966) allows for a greater degree of accuracy in assessing the morphological condition of the organ of Corti. In serial sectioning, as Bredberg & Hunter Duvall (1975) have pointed out, every 10th section is generally stained and inspected which means that small lesions may possibly be missed. No such accuracy exists in our data. Lesions as small as 37  $\mu\text{m}$  (Fig. 5a) incorporating only 8 OHCs

are clearly visible and the absence of only one hair cell is easily detectable. In addition the high resolution of SEM will reveal surface structural abnormalities which may go undetected with light microscopy. We have assumed that obvious abnormalities in cuticular plates and stereocilia imply abnormal functioning of the hair cell.

Several important points emerge from our results. Firstly the excellent correlation between location of maximum hair cell loss and frequency of maximum loss of  $N$  threshold sensitivity provides an additional validation of the place-frequency map of the guinea pig cochlea, even though our lesions were confined by necessity to a fairly restricted region of the basal cochlear spiral. The most complete place frequency maps have hitherto been derived from basilar membrane mechanical measurements (Wilson & Johnstone 1975) and from spiral ganglion recordings (Robertson & Manley 1974; Johnstone 1977) both methods showing substantial agreement. From data provided by Wilson and Johnstone (1975) the equation

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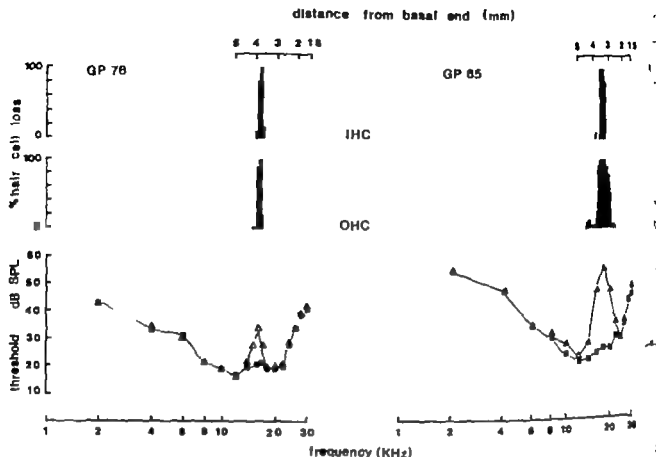


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end of the basilar membrane and then aligned with the lower frequency scale according to the place frequency map of Wilson & Johnstone (1975) and Robertson & Manley (1974).

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transmission electron microscopy (TEM) may reveal ultrastructural abnormalities within the apical cell body. No studies of this nature were carried out in this experiment.

## DISCUSSION

The sequence of events following mechanical disruption of the organ of Corti and subsequent degeneration of the receptor cells is a yet obscure. Introduction of a 70  $\mu\text{m}$  glass probe through the cochlear partition in this study produced loss of hair cells for distances of up to 1.1 mm. One possible mechanism which may result in hair cell degeneration is a bruising of the cochlear fluids, perilymph and endolymph. Disruption of the integrity of both Reissner's membrane and the cochlear partition in other studies has produced loss of receptor cells similar to that found in this series of animals (Lawrence, 1966; Duvall & Rhodes, 1967; Duvall et al. 1969). In our experiments V thresholds were not followed for more than 30 min after piercing the organ of Corti and consequently it was not possible to assess the final extent of the hearing loss until post-recovery electrocochleograms were measured. Because the final lesion was in all cases much more extensive than the size of the probe, pure mechanical disruption of the organ of Corti seems a remote cause of the final loss of the receptor cells. In any case this method has enabled spatially restricted reproducible loss of hair cells to be correlated with the resultant deficiencies in the N electrocochleogram.

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are clearly visible and the absence of only one hair cell is easily detectable. In addition the high resolution of SEM will reveal surface structural abnormalities which may go undetected with light microscopy. We have assumed that obvious abnormalities in cuticular plates and stereocilia imply abnormal functioning of the hair cell.

Several important points emerge from our results. Firstly the excellent correlation between location of maximum hair cell loss and frequency of maximum loss of N threshold sensitivity provides an additional validation of the place-frequency map of the guinea pig cochlea even though our lesions were confined by necessity to a fairly restricted region of the basal cochlear spiral. The most complete place-frequency maps have hitherto been derived from basilar membrane mechanical measurements (Wilson & Johnstone 1975) and from spiral ganglion recordings (Robertson & Manley 1974; Johnstone 1977) both methods showing substantial agreement. From data provided by Wilson and Johnstone (1975) the equation

$$f = 43e^{-0.077x} \quad (1)$$

can be derived where  $f$  is the cut-off frequency for the basilar membrane tuning curve and  $x$  is distance from the basal end of the basilar membrane. Excellent agreement was found in our present data between the frequency of maximum loss in the N electrocochleogram and the location of hair cell loss when these were aligned according to the above equation. Thus eq. (1) appears to reflect accurately the spatial representation of frequency in auditory nerve fibres. The present N data, together with recent spiral ganglion cell recordings (Robertson et al. in preparation) from our laboratory suggest that the primary nerve fibres at a given location respond best to the frequency corresponding to the high frequency cut-off rather than the peak frequency of the basilar membrane tuning curve at that location (Wilson & Johnstone 1975). This may have im-

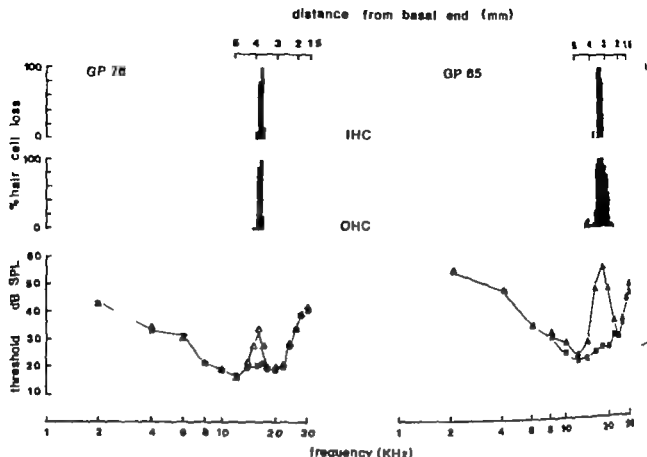


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curred on opening scala tympani and corpus of Capus pierced in both cases.  $\Delta$  Post-recovery N<sub>1</sub> threshold.

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authors (Huang et al 1976, Dallos & Harris 1978, Robertson & Johnstone 1978) that outer hair cells may in some as yet obvious way be necessary for the normal functioning of the afferent nerve fibres which innervate the inner hair cells.

## ACKNOWLEDGEMENTS

This work is supported by grants from the Australian earth Grants Council and by the Swedish Medical earth Council, project no. 3542. G. Bredberg was supported by a Rasmussen Foundation Visiting Professorship. Robertson is Queen Elizabeth II Fellow. A. Cody is overseas earth Postgraduate Student.

## ZUSAMMENFASSUNG

Die getrennten Verletzungen wurden im Cortischen Organ der Meeresschnecken Cochlea erzeugt, indem Frep- und Hochfrequenzschallwellen direkt mechanisch auf die Cochlea einwirkten. Der elektrophysiologische Zustand der Cochlea wurde durch N-Elektrococleographie eingeschätzt und der Verlust an Rezeptorzellen mit Abtastung und Elektronenmikroskop bestimmt. Die Hauptbefunde waren: 1) Vorübergehende Überstimulation zwischen dem Fokussieren von Haarzellenverlusten und 2) Frequenz- und Hochfrequenzschallwellenwechsel im N-Elektrococleogramm. 2) Die räumliche Ausdehnung der mechanischen Verletzung scheint wichtiger zu sein als die Gewebeschädigung der verlorenen Haarzellen für die Bestimmung der Höhe des Verlustes an N-Empfindlichkeit. 3) Haarzellenverluste, die sich über nur 77 µm ausdehnten, konnten als bedeutende Veränderungen der N-Empfindlichkeit festgestellt werden. Diese Resultate betonen weiterhin die Genauigkeit und Brauchbarkeit des N-Elektrococleogramms für die Einschätzung der Schallübertragung der Cochlea. 4) Verletzungen, die sich auf den Verlust der äußeren Haarzellen betrafen, beinhalten auch starkere Erhöhungen der N-Schwelle.

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portant implications for the mechanism of frequency coding in the cochlea. It is worth noting that our results contrast with numerous studies (outlined by Bredberg & Hunter-Duvar 1975) in which the absence of an accurate place frequency map has hampered attempts to correlate hearing loss and cochlear pathology.

The  $N_1$  electrocochleogram therefore appears to reflect accurately the functional state of the organ of Corti in that structural damage to a given region is consistently manifested by a deficit in  $N_1$  sensitivity in the expected frequency range. Indeed our results highlight the convenience and elegance of the  $N_1$  electrocochleogram in assessing cochlear pathology at least in the range 2–30 kHz as used in this study.

Reproducibility of the  $N_1$  threshold measurement and the ability to rapidly test many frequencies contrasts with behavioural audiograms where the time-consuming nature of testing inherent variability in response and limited frequency steps greatly limit accuracy. Other recent studies (Johnstone *et al* 1978; Dallos *et al* 1978) also attest to the usefulness of the electrocochleogram and its potential importance as a diagnostic tool in clinical assessment of hearing loss in human patients.

Other features of the correlation between hair cell loss and  $N_1$  deficits are also interesting. The data suggest that it is the spatial distribution of hair cell loss rather than the number of hair cells lost *per se* which determines the resulting magnitude of the  $N_1$  deficit. This presumably reflects the spatial spread of the excitation pattern to hair cells. A wide lesion means that sound pressure at a particular frequency must be raised to a greater extent to excite distant functional hair cells than in the case of a narrow lesion where functional hair cells are closer spatially to the frequency of maximum excitation. In addition two features of the  $N_1$  notch in the lesioned animals suggest that hair cells on the border of the lesion are functionally abnormal though their surface ultrastructure does not differ from that of non-

lesioned cochleas. Firstly the width of the  $N_1$  notch is considerably larger in many cases than the frequency width of the anatomical lesion. Secondly if the lesions were ideal, the  $N_1$  notch would have high and low frequency slopes of the same order of magnitude as single unit tuning curves (i.e. greater than 100 dB/octave (Kiang 1965; Evans 1972; Robertson & Manley 1974)). No doubt the fact that the edges of our lesions are somewhat diffuse contributes to the findings of notch slopes of the order of 50 dB/octave but once again it is likely that the presence of abnormally functioning hair cells which appear anatomically normal leads to this reduction in the ideal steepness of the notch. It is possible that longer survival times are required to allow degeneration of hair cells which have not yet begun to exhibit their occult structural abnormalities.

In many of our animals we are unable to exclude the possibility that damage to structures other than hair cells (i.e. supporting cells and nerve fibres) may contribute to the  $N_1$  deficit. We have made no attempt to quantify alterations in structures other than hair cells.

Undoubtedly the most intriguing results are from animals which showed  $N_1$  sensitivity losses on opening the scala tympani (Fig. 6). In all these animals (4 in number) only outer hair cells were missing yet  $N_1$  deficits showed the same characteristics although more pronounced as in deliberately lesioned cochleas with mixed inner and outer hair cell loss. The mechanism for these inadvertent lesions is obscure. That it could be due to the noise and vibrations when drilling the holes in the scala tympani wall does not seem probable as there was little recovery in the final  $N_1$  electrocochleogram (mean 2.5 dB S.D. 5.3 ears).

The results are clear: loss of even just one row of OHCs with apparently normal remaining OHCs and completely intact inner hair cells is sufficient to produce up to a 40 dB peak change in  $N_1$  threshold. Since the majority of nerve fibres emanate from IHCs (Spoendlin 1972) it appears as already suggested by vari-

## PENETRATION OF THE COCHLEAR ENDOST BY THE FIBROUS COMPONENT OF THE OTOSCLEROTIC FOCUS

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Under the role of the third "fibrous" component of the otosclerotic focus in penetrating and absorbing the cochlear endost is documented. An otosclerotic focus with a fibrous-praxiomatous component shows equal power as spongiotic or sclerotic components producing degeneration resulting in rotary-formed absorption of the endost opening access to the soft-tissue containing regions of the cochlea.

Penetration of the cochlear endost can be achieved not only by the two components of the otosclerotic focus: the spongiotic and sclerotic but also by a third: the fibrous-praxiomatous mass which is surrounded by the two bony portions (Kelemen 1970). This mass is not merely filler material: its destructive and penetrating power equals that of the two bony components.

The space occupied by this mass can be extensive, comprising a considerable part of the focus. This space has been identified by various names such as: fissula ante and post fenestram, resorption foci, vascular space and acellular space. When the third component surrounds the corner of the stapedial footplate it gives rise to what is called a "loose stapes" or a "floating footplate".

Mayer (1923) considered the wide spaces filled with fibrous marrow to be one of the three forms of otosclerotic organization. Johnson, Hawkins and Luthincum (1973) concluded that it was possible for a stapedial ankylosis to exist without an ankylotic tapes: a complete immobilization due to a fibrous mass surrounding the footplate rather

than to spongiotic or sclerotic bone overgrowing the angle of the footplate.

While discussion of Stebenmann's (1879) theory of the poisonous production of the otosclerotic focus continues (Chevance et al. 1970) it is generally agreed that the focus that breaches the cochlear endost and enters the cochlear lumen, reaching the soft tissues, causes functional damage. Replacement of the endost by the focus may result in atrophy of the spiral ligament and of the basilar membrane (Friedmann 1974).

This paper demonstrates the role of the third component at a region of strategic significance: the boundary of the cochlear layers of endochrondrium and endost.

### MATERIALS

Upon examination of 748 temporal bones from 137 patients with otosclerosis in one or both ears, 99 were found with the focus reaching the cochlear endost. All temporal bones were sectioned in the horizontal plane and stained with hematoxylin and eosin. This paper discusses and illustrates 11 examples from the series.

### DISCUSSION

In many cases the otosclerotic invasion stopped short of the endost. Hough (1964) referred to an invasion that stopped short of the stapedial ringband as "hesitation". In this type of invasion it can be assumed that the cochlear endost might be exhibiting a form of self-defense.

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Fig. 4. Wedge-shaped replacement of the perist by sclerotic portion advancing by fingers.  $\times 82$ .

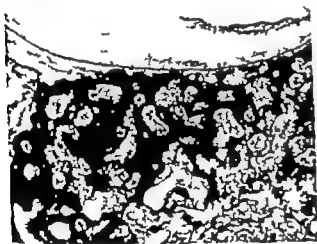
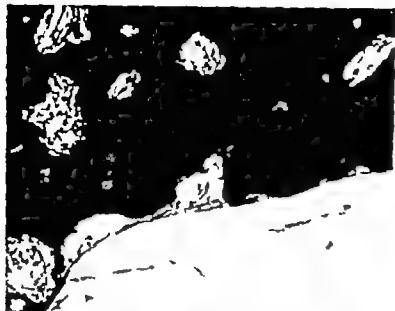


Fig. 5. Replacement of endost, in rosette formation, by spongiotic foci.  $\times 82$ .



Fig. 6. Replacement of endost by foci rich in fibrous component.  $\times 52$ .





*Fig 1* Endost destroyed by progressing lesion  $\times 172$ .



*Fig 2* Fissure (crack) produced by pressure of the advancing focus rich in fibrous component  $\times 90$ .



*Fig 3* Fissure (crack) caused by pressure of advancing focus extending to the non-otosclerotic osseous mass in the scala.  $\times 82$ .

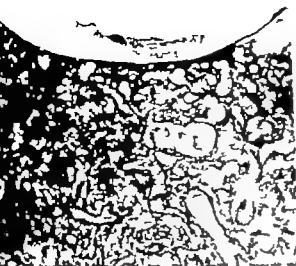


Fig 10 Wedge-shaped replacement, in rotary formation, of endost by focus rich in fibrous component.  $\times 52$ .



Fig 11 Wedge-shaped replacement, in rotary formation, of endost by focus rich in fibrous component.  $\times 105$

As demonstrated by many cochlear fractures with intact endost (Kelemen 1944) the endost certainly has resisting power. According to Brunner (1957) otosclerotic bone approaching the endosteum becomes atrophic. Whether a sclerosis of the endost means defence or vulnerability is questionable. Antoli-Candela et al (1977) followed the involvement through the cochlear wall across the spiral ligament to the basilar membrane. Friedmann (1974) pointed to atrophy of the spiral ligament and rupture of the basilar membrane. According to Linthicum et al (1975) narrowing of the

cochlear lumen and distortion of the basilar membrane are caused by an otospongiotic focus that impinges on the cochlear wall. Benitez & Schuknecht (1964) showed that otosclerosis extending to the endosteum is accompanied by atrophy of the spiral ligament.

The physical force of the focus advancing to the endost is first revealed by a crack or fissure in the endost, as illustrated in Kelemen & Linthicum (1969). Penetration of the endost by the spongiotic or sclerotic portion of the focus is usually wedge shaped as shown by Manasse (1917).

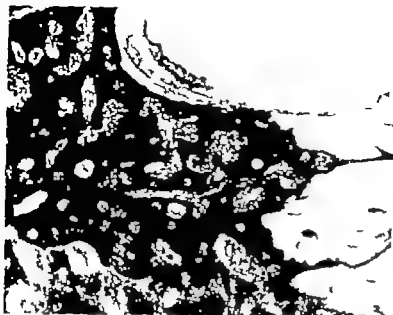


Fig. 7 Replacement of endost by focus rich in fibrous component,  $\times 52$ .

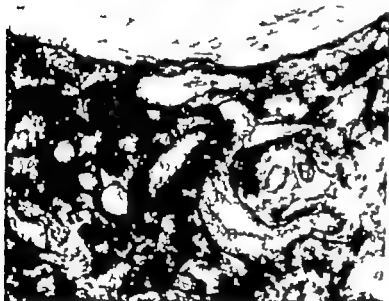


Fig. 8 Replacement with uneven surface of the endost by focus rich in fibrous component  $\times 110$ .



Fig. 9 Replacement in rosary-like formation of endost by focus rich in fibrous component  $\times 82$ .

# THE DIAGNOSIS OF NEGATIVE MIDDLE EAR PRESSURE IN CHILDREN

## *The Accuracy of Symptoms and Signs Assessed by Tympanometry*

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**Abstract.** Over a period of one year a standard 35° child-  
drive at random in order to determine whether or not  
diagnosis of negative middle ear pressure can be made  
reliably without using tympanometry. Each child was  
tested repeatedly by screening audiometry at 20 dB and  
by tympanometry and was examined once by otoscopy.  
Information about upper respiratory tract infection,  
feelings of oppression in the ears, and the parents  
opinion of the child's hearing ability were obtained from  
questionnaires. Otoscopy correlated poorly with the  
tympanometry. Both screening audiometry and the  
parents' opinion of the child's hearing ability are more  
reliable sensors. It is concluded that tympanometry is  
an absolutely necessary tool in the clinical diagnosis of  
negative middle ear pressure.

doctors may claim that the clinical diagnosis  
of middle ear pathology (i.e. of negative pres-  
sure) can be made with sufficient reliability  
without tympanometry.

In order to evaluate the truth of this assump-  
tion it is necessary to examine what is meant  
by a diagnosis. For this purpose the elements  
of the clinical diagnosis may be defined as  
symptoms and signs. Symptoms are those con-  
comitants of the disease or clues to the disease  
about which the patient can give positive state-  
ments, while signs may be observed or meas-  
ured by the physician. Thus the clinical diag-  
nosis may be analysed by evaluating each  
symptom and sign separately but without ana-  
lysing the complex interactions of all elements  
together.

This principle was applied to an epidemio-  
logical study conducted during 1977 of nega-  
tive middle ear pressure in a randomly se-  
lected group of children. The signs of middle  
ear disease were evaluated from tympano-  
metric, audiometric and otoscopic observa-  
tions and some corresponding symptoms of  
middle ear pathology were obtained from  
questionnaires answered by the parents.

## METHODS

The study involved children from six schools  
in Vejle, which is an industrial town of about  
50 000 inhabitants. From 16 classes a total of  
357 children were selected, all of whom were

negative middle ear pressure which is the re-  
sult of an impaired function of the Eustachian  
tube occurs very frequently in children (Ren-  
vall et al 1978; Fiellau-Nikolajsen et al  
1977). If it is persistent, secretory otitis media  
(SOM) may develop, a condition which is  
characterized by a nonpurulent middle ear ef-  
fusion. Because the results of today's treat-  
ment of SOM are not satisfactory, as stated by  
Tos & Poulsen (1976), early diagnosis and  
treatment of this problem, if possible before  
the effusion has developed, would be desirable.

Impedance audiometry or tympanometry  
has gained widespread use in otology as a  
means of documenting the middle ear pressure  
objectively. The frequency with which this  
technique is used seems to vary considerably  
among otologists, and its use is very limited  
among colleagues in other specialties such as  
pediatricians and family doctors. Thus some

The most conspicuous form of replacement of the endost by the focus was called fragmentation by Kelemen & Linthicum (1969). Before disappearing, the endosteal layer is broken into a chain of beads resembling a rosary (Rüedi 1969, Siebenmann 1899, Friedmann 1974). Fragmentation of fibrils producing a rosary like figure was seen by Chevance under the electron microscope.

It is shown that otosclerotic foci rich in fibrous elements have the same power as the spongiotic and sclerotic components producing fragmentation resulting in absorption of the endost.

### ZUSAMMENFASSUNG

Die „dritte“ „fibrös-glomerulöse“ Komponente des otosklerotischen Fokus hat dieselbe Fähigkeit, das Endost zu durchbrechen wie die spongiöse oder sklerotische Komponente mit Eröffnung als Zugang zu dem inneren Raum der Schnecke.

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Table III The clinical validity of the symptoms and signs presented in Tables I and II as indicators of middle ear pathology

| Middle ear pressure indicating threshold of pathology (mmH <sub>2</sub> O) | Sensitivity % |      | Specificity % |      | Relative liability |      |
|--|---------------|------|---------------|------|--------------------|------|
|  | -160          | -240 | -160          | -240 | -160               | -240 |
| Upper respiratory tract infection  | 13.1          | 14.8 | 91.1          | 90.5 | 1.57               | 1.77 |
| Otoscopic  | 54.7          | 65.3 | 64.1          | 63.6 | 1.16               | 3.00 |
| Oppression in the ears   | 67.5          | 67.5 | 97.6          | 97.3 | 3.01               | 2.79 |
| Hearing ability  | 23.2          | 25.3 | 93.3          | 91.7 | 4.19               | 3.76 |
| Screening audiogram  | 1.9           | 27.2 | 95.9          | 95.0 | 6.62               | 7.07 |

Answers to be answered by the parents were distributed by the school teachers a few days before each measurement. The questionnaire was answered by checking one answer with a maximum of three possible answers. For this analysis a distinction was made only between negative and positive answers. The number of questionnaires was 1349; thus for 86% of the ears tested data from a corresponding questionnaire was available for comparison.

## RESULTS

The presence or absence of symptoms of middle ear problems as determined by the questionnaires is presented in Table I. The parents were asked if during the last few days the child had shown signs of upper respiratory tract infection and if there had been any complaint of a feeling of oppression in the ears. Usually the parents gave their opinion of the child's hearing ability as assessed in the everyday situation in the home. The questions did not specify if the right, the left or both ears were involved and therefore the answers were compared with the corresponding mean value of the middle ear pressure of both ears. On the primary the signs assessed by otoscopy and by the screening audiogram were recorded for each ear separately as shown in Table II. In Table I and Table II the proportions of negative symptoms and signs seen in children with and in those without disease were compared by the  $\chi^2$ -square test. It appears from the

results of the  $\chi^2$ -square analysis that a middle ear pressure of less than -240 mmH<sub>2</sub>O is not statistically related to the occurrence of upper respiratory tract infection.

Sensitivity, specificity and relative liability of each symptom and sign were computed in the further analysis of the results. The sensitivity of the test is the ratio of positive tests in all children with the disease (true positives or TP) to the total number of children who actually have the disease when disease is defined as a middle ear pressure below an indicated threshold (-160 mmH<sub>2</sub>O or -240 mmH<sub>2</sub>O).

The specificity is the ratio of negative tests in all children without disease (true negatives or TN) to the total number of children without disease. The higher the values of sensitivity and specificity the more reliable is the symptom or sign as an indicator of disease. The complement of TP is false-positives (FP) and that of TN is false-negatives (FN). Obviously a diagnostic method with a high value of TP/FP is a good discriminator of disease while a method with a high value of TN/FN is a good discriminator of health. In order to compare the effectiveness of different tests as measures of health or disease both of these values must be considered.

For practical purposes the formula  $(TP \times TN) / (FP \times FN)$  may be used as a unified measure in the comparison. It expresses the relative liability of children with disease to show a positive indication of disease compared with the tendency of children free of disease to show positive indications of dis-

Table I The occurrence of appropriate symptoms in groups of children in whom the mean middle ear pressure of both ears was higher or lower than thresholds of  $-160$  mmH<sub>2</sub>O and  $-240$  mmH<sub>2</sub>O

| Mean middle ear pressure in both ears ( $-$ mmH <sub>2</sub> O) | Upper respiratory tract infection |            |       | Oppression in the ears |            |       | Hearing ability |            |            |
|---|-----------------------------------|------------|-------|------------------------|------------|-------|-----------------|------------|------------|
|   | Yes                               | No         | % Yes | Yes                    | No         | % Yes | Impaired        | Normal     | % Impaired |
| 0-159   | 97                                | 1 000      | 8.8   | 1                      | 840        | 4     | 74              | 1 036      | 6.7        |
| 160-400   | 28                                | 184        | 13.0  | 10                     | 133        | 7.0   | 49              | 16         | 3          |
| $\chi^2$ square   |                                   | $p < 0.05$ |       |                        | $p < 0.01$ |       |                 | $p < 0.01$ |            |
| 0-239   | 117                               | 1 109      | 9.1   | 77                     | 974        | 8     | 101             | 1 13       | 8.3        |
| 240-400   | 13                                | 75         | 14.8  | 4                      | 49         | 7.5   | 22              | 83         | 25.3       |
| $\chi^2$ square   |                                   | $p > 0.05$ |       |                        | $p < 0.05$ |       |                 | $p < 0.01$ |            |
| Total of answers  | 125                               | 1 184      | 9.5   | 31                     | 973        | 3.1   | 123             | 1 188      | 9.4        |

about 7 years old at the time of the study. A few children with known sensorineural hearing losses were excluded from the study but those who had been treated for SOM were included.

Otologic evaluation of the children was performed in school five times during 1977. Each examination consisted of a screening audiogram to detect hearing losses greater than 20 dB at six frequencies and impedance audiometry to measure the middle ear pressure. The Danplex audiometer (TDH 39 headphones) and electroacoustic impedance bridge (220 Hz probe tone) (Model DI 75) were used to determine the presence of a hearing loss and to measure middle ear pressure. The middle ear pressure was determined from the air pressure in the external ear canal at the time when the electroacoustic bridge showed minimum im-

pedance (maximum mobility) of the tympanic membrane. In the absence of a maximum point for tympanic membrane mobility the middle ear pressure was recorded as  $-400$  mmH<sub>2</sub>O. The children who failed this screening procedure according to our criteria were referred to the Hearing Clinic which is part of the Otolaryngology Department in Vejle Hospital. Of the 352 children screened several times 21 children had repeated ear testings in the soundproof room with simultaneous determinations of middle ear pressure.

The present report is concerned with the screening procedure. According to the design of the study 1760 pairs of ears could have been tested but for various reasons (mainly absence from school on the day of testing) only 1577 tests were recorded. The question

Table II The occurrence of appropriate signs in groups of children in whom the middle ear pressure was higher or lower than thresholds of  $-160$  mmH<sub>2</sub>O and  $-240$  mmH<sub>2</sub>O

| Middle ear pressure ( $-$ mmH <sub>2</sub> O) | Otoscopy |            |            | Screening audiogram |            |            |
|---|----------|------------|------------|---------------------|------------|------------|
|   | Abnormal | Normal     | % Abnormal | Abnormal            | Normal     | % Abnormal |
| 0-159   | 63       | 470        | 35.9       | 91                  | 15         | 4.1        |
| 160-400                                       | 75       | 6          | 54.7       | 84                  | 300        | 21.9       |
| $\chi^2$ square                               |          | $p < 0.01$ |            |                     | $p < 0.01$ |            |
| 0-239   | 289      | 506        | 36.4       | 122                 | 310        | 5.0        |
| 240-400                                       | 49       | 6          | 65.3       | 53                  | 14         | 7          |
| $\chi^2$ square                               |          | $p < 0.01$ |            |                     | $p < 0.01$ |            |
| Total of observations                         | 318      | 53         | 38.9       | 174                 | 457        | 6.7        |

line analysed the prognostic abilities of more specialized methods of otoscopy such as the use of the operating microscope (Axelsson & Lewis 1976) which showed a very high accuracy rate. Roeser et al (1977) compared the assessments of an experienced otologist and a pediatrician each of whom examined the children with a pneumatic otoscope and impedance measurements. They found poor agreement between the results of otoscopy and impedance measures as well as poor inter-examiner agreement.

The best relative liability was found concerning the parent's assessment of the child's hearing ability and concerning the results of screening audiometry; however the sensitivity of these assessments was only about 25%. The reports in the literature on screening audiometry are difficult to compare as the criteria for abnormality vary. In general the results from our study seem to be in agreement with those of most other investigations (Berry et al. 1975; Brooks, 1973).

Only about one-fourth of the measured pathological middle ear pressures were accompanied by positive symptoms and signs. As appears from Fig. 1 as the 3.7% of the measurements with both features vis-à-vis the total of 15.4% of the measurements that showed pathological middle ear pressures. So this simplified analysis shows that the symptoms and signs which we evaluated are not good prognosticators of negative middle ear pressure below  $-160 \text{ mmH}_2\text{O}$  besides being a reliable precursor of SOM: a pressure in that age should be considered a possible hearing handicap (Likholdt et al. 1979). Accordingly the recognition of a negative middle ear pressure is important. This study shows the limited value of simple otoscopy in this diagnosis. This is especially significant because of the importance attributed to this procedure by otologists. In fact, more reliable information may be obtained by asking parents about their child's hearing ability or by recording a screening audiogram than may be obtained by otoscopy.

This study confirms the conclusion of many other investigators that impedance audiometry is an absolutely necessary tool in the clinical diagnosis of negative middle ear pressure.

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## ZUSAMMENFASSUNG

In einer Periode von einem Jahr haben wir 357 zufällig ausgewählte Kinder untersucht, um herauszufinden, ob es möglich ist, eine zuverlässige Diagnose eines negativen Mittelohrdrucks zu stellen ohne Tympanometrie zu verwenden. Jedes Kind wurde wiederholt durch Screening Audiometrie bei 20 dB sowie bei Tympanometrie untersucht und wurde einmal durch Otoskopie untersucht. Anknüpfte über Infektionssymptome in dem oberen Respirationstrakt das Gefühl eines Drucks in den Ohren und die Beurteilung der Eltern über das Gehörvermögen des Kindes wurden durch Fragebogen erhoben. Otoskopie bildete eine schlechte Korrelation mit Tympanometrie während Screening Audiometrie und die Beurteilung der Eltern über das Gehörvermögen des Kindes ein zuverlässiges Maß anzeigte. Daraus folgert, daß Tympanometrie ein absolut notwendiges Gerät der klinischen Diagnose eines negativen Mittelohrdrucks ist.

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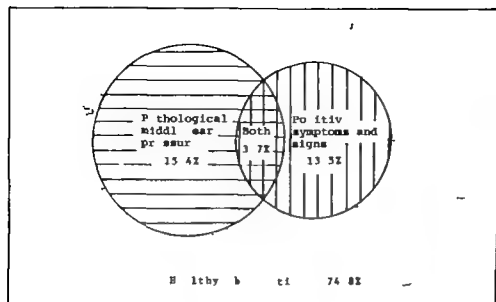


Fig 1 Average occurrence of pathological middle ear pressure (a threshold of  $-160$  mmH<sub>2</sub>O is used to designate pathology) and/or positive symptoms and signs of pathology in 352 school-children chosen at random. Single measurements and mean values have been combined in this diagram.

case. The higher the value of the relative liability the more useful (i.e. reliable) is the diagnostic method (Abrahamson 1974). In Table III the symptoms and signs are ranked according to their relative liability.

Fig 1 gives a general idea of the relationship between pathological middle ear pressures and positive symptoms and signs of this condition. In this Venn diagram the intersection of two sets constitutes a small subset where middle ear pathology is associated with positive symptoms and signs, suggesting that tympanometry is a prerequisite to identify negative middle ear pressure.

## DISCUSSION

In this study the analysis of middle ear pathology as a diagnostic problem was made by simplifying the problem. Thus any projected use of this system to diagnose middle ear pathology in daily clinical practice should be done carefully. Furthermore questionnaires were used as the source of patient information while interviews are a very important part of most clinicians' assessment and subsequent diagnosis. In some respects questionnaires provide information which is superior to that gleaned in an interview, as a larger number of less biased observations may be tabulated

from questionnaires, and both the parents and the examiners were unaware of the results of other data gathering tests when answering the questionnaires or performing the measurements.

As shown by Table III the clinical validity of the symptoms and signs as indicators of disease does not vary much with the cutoff value for pathological middle ear pressure. For instance the symptom of oppression in the ears is of limited value in identifying pathology, and the presence of an upper respiratory tract infection is of no value in predicting middle ear pathology, which also appears from Table I. Otoscopy is a sensitive method but its low specificity reduces its clinical value. It should be noted that otoscopy was performed by experienced otologists but without knowledge of the actual middle ear pressures. Otoscopy is in fact the procedure most often used by doctors to diagnose middle ear disease; nevertheless we found only one report in the literature which described a comparison of otoscopy with other methods of assessing middle ear function (Ferrer 1974). In this report it was not specified whether the otologist knew the actual middle ear pressure but it confirmed the sensitivity of about 50% found in our study while reporting a higher specificity rate of about 80%. Some authors

re-analysed the prognostic abilities of more realized methods of otoscopy such as the use of the operating microscope (Axelsson & Wik, 1976) which showed a very high accuracy rate. Roeser et al (1977) compared the assessments of an experienced otologist and a layman each of whom examined the child with a pneumatic otoscope and impedance measurements. They found poor agreement between the results of otoscopy and impedance measures as well as poor inter-examiner agreement. The best relative validity was found concerning the parent's assessment of the child's hearing ability and concerning the results of screening audiometry; however the sensitivity of these assessments was only about 25%. Reports in the literature on screening otoscopy are difficult to compare as the criteria for abnormality vary. In general the results from our study seem to be in agreement with those of most other investigations (Cryer et al 1975; Brooks 1973). Only about one-fourth of the measured pathological middle ear pressures were accompanied by positive symptoms and signs (as appears from Fig. 1 as the 3.7% of the measurements with both features *vis-à-vis* a total of 15.4% of the measurements that showed pathological middle ear pressures. So a simplified analysis shows that the symptoms and signs which we evaluated are not good prognosticators of negative middle ear pressure below  $-160 \text{ mmH}_2\text{O}$  besides being a reliable precursor of SOM, a pressure in that range should be considered a possible hearing handicap (Laidholdt et al 1979). Accordingly the recognition of a negative middle ear pressure is important. This study shows the added value of simple otoscopy in this diagnosis. This is especially significant because of the importance attributed to this procedure by otologists. In fact more reliable information may be obtained by asking parents about their child's hearing ability or by recording a screening audiogram, than may be obtained by otoscopy.

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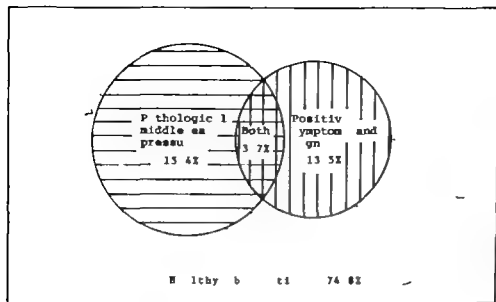


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Fig 3 Scintigram of patient II presented according to Fig 1. Increased accumulation in the left mastoid region.

strated an abnormally high accumulation of the isotope corresponding to the right mastoid region and upper lateral part of the right external meatus which areas were left intact at earlier surgery (Fig 2). The patient was then irradiated with 60 Gy in fractionated doses. A second scintigraphy 2 weeks after the last irradiation dose showed an increase of the pathologic isotope accumulation. The patient died from recurrent carcinoma of the ear and bronchopneumonia in March 1977.

#### Patient II

A man aged 75 without history symptoms or signs of chronic otitis. In 1961 a basal cell carcinoma of the skin developed behind the left auricle. The tumour was excised, but from 1966 to 1976 local recurrences appeared and were excised five times. In 1972 the peristoma of the mastoid process was excised in connection with the skin excision but the bone was left intact. In July 1976 a 4×4 cm tumour was palpated behind the ear under the skin and against the bone. Histology demonstrated a recurrence and the histopathological diagnosis was changed to squamous cell carcinoma. Radiography did not demonstrate any destruction but tomography demonstrated a destruction of bone under the tumour probably not explained by the excision of peristoma in 1972.

Scintigraphy was performed on the same day as the tomography and revealed an abnormally high radionuclide accumulation in the

left mastoid region corresponding to the tumour recurrence and the destruction demonstrated by tomography (Fig. 3). The patient was irradiated with 40 Gy in fractionated doses. A second scintigraphy 2 weeks after the last irradiation dose demonstrated an increase in the pathologic isotope accumulation. The patient was operated by radical mastoidectomy but in February 1979 again a recurrence was verified by histology.

#### Patient III

A woman aged 72 with symptoms of chronic inflammation of the left middle ear since childhood with periods of excretion from the ear. A chronic central perforation had been present at least for the last 5 years, but the ear had been dry during this period. Since December 1975 excretion and slight pain had been present in the ear. At examination the internal parts of the external meatus were filled with abnormal soft tissues possibly engaging the middle ear. No symptoms of cochlear or vestibular engagement were present. Histologic examination of small biopsies revealed a highly differentiated squamous cell carcinoma. Ordinary radiography demonstrated a dense cell system and absence of bone details in the floor of the external meatus.

Tomography of the ear also demonstrated the bone destruction of the floor of the external meatus. The hearing bones could not be identified and the air space of the middle ear

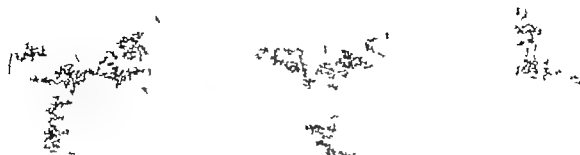


Fig 1  $^{99}\text{TcDP}$  bone scintigram of the facial skeleton of a control subject. RL, right lateral projection LL, left lateral projection AP antero-posterior projection

general skeletal survey because of early mammary cancer. They did not have any affections of the ear regions nor did they show any symptoms or signs of metastases in the axial skeleton or skull. All 10 control cases had a symmetrical distribution of  $^{99}\text{TcDP}$  in the facial skeleton and in the temporal bones (Fig 1).

#### Patient 1

A man aged 73 complained of excretion from his right ear since December 1975. There was no history of chronic ear affection. Clinical examination in March 1976 revealed a tumour 1 cm in diameter in the central part of the auricula and engaging the outer part of the external meatus. Ordinary radiography in March 1976 demonstrated a slightly decreased amount of air in the mastoid process on the affected side but no bone destruction. How-

ever tomography revealed destruction in the upper part of the external meatus.

An operation was performed in April 1976 and the auricula, the parotid gland and associated soft tissues were excised together with the cartilaginous part of the external ear. The mastoid process and the bony part of the external meatus were left intact. Histologic examination demonstrated a highly differentiated squamous cell carcinoma growing close to but not into the excision margin against the inner part of the external meatus. In July 1976 a facial palsy appeared on the operated side and specimens taken for further histologic examination demonstrated carcinoma tissue in the bony part of the external meatus. Tomography of the ear in August 1976 did not demonstrate any change of structure beyond those described in March 1976. Scintigraphy demon-

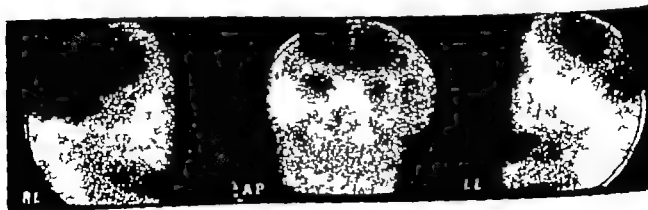


Fig 2 Scintigram of patient 1 presented according to Fig. 1. Increased accumulation in the right mastoid and outer meatal area.



Fig 6 Scintigram of patient IV presented according to Figs. 1 and 5. Normal accumulation in both temporal areas. No side difference in AP projection.

ndings at operation could not demonstrate massive growth of tumour beyond in the walls

the external meatus but possibly in the rotympanon region between this area and the carotid canal. Seven months after surgery recurrence appeared in the operation cavity despite treatment with cytostatic drugs the patient died from the cancer in 1977.

#### Case IV

A man aged 69 with recurrent excretion from his right ear for 10 years. Examination in 1976

revealed a basal cell cancer of the external meatus engaging the meatus as well as the cranial part of the ear drum. Tomography of the ear did not demonstrate any bone destruction. Scintigraphy was performed in connection to the roentgen examination and demonstrated accumulation of  $^{99m}\text{Tc}$ -DP in both ear regions which were assessed as normal and without side difference (Fig. 6).

No radiotherapy was given. At operation the tumour was found to be growing along the bony walls of the external meatus but no bone



Fig 4 Scintigram of patient III presented according to Fig 1 Increased accumulation in the entire pyramid Central area of low intensity

was replaced by soft tissue No pathologic changes of the inner ear region were demonstrated

Scintigraphy was performed in connection to the roentgen examination and an abnormal accumulation of  $^{99}\text{Tc}^{\text{m}}$ DP corresponding to the entire petrous part of the left temporal bone was demonstrated Scintigraphy demonstrated a lower intensity in the central part of the tem-

poral bone than in the surrounding parts (Fig 4) The abnormal accumulation of  $^{99}\text{Tc}^{\text{m}}$ DP was better visualized in the oblique lateral projection (L O L) than in the lateral projection (L-L) (Fig 5) The patient was then irradiated with 40 Gy given in fractionated doses against the area. A second scintigraphy 7 weeks after the first irradiation dose demonstrated an increase of the pathologic isotope accumulation



Fig 5 Scintigram of a control subject (top) and of patient III (bottom) (oblique lateral projections, ROL, right oblique lateral projection, LOL, left oblique lateral projection) The accumulation in affected left temporal bone more easily visualized. Compare Fig 4

## Patient V

A woman aged 40 suffering from external otitis of the left ear since February 1979. In July a biopsy was taken demonstrating a medium-high differentiated cancer of the ear and the patient was referred for surgery. Radiography in August 1979 demonstrated a slightly increased attenuation in the lateral part of the mastoid cell system of the affected ear. Polytomography revealed a destruction of the lower part of the external meatus as well as of the temporomandibular joint fossa and the anterior part of the mastoid process.

Scintigraphy demonstrated a definitely increased accumulation of  $^{99m}\text{Tc}$ -DP in corresponding regions but the area affected according to the scintigraphic results was larger than according to the radiographic results (Fig. 8).

The patient was operated one week later and the tumour was found to be growing extensively in the temporal bone and into the parotid region.

## DISCUSSION

Five patients (I, II, III, V and VI) with verified squamous cell carcinoma of the ear and bone destruction demonstrated by tomography had abnormally high accumulation of  $^{99m}\text{Tc}$ -DP in the temporal area. One patient with a basal cell carcinoma (IV) had no demonstrable bone destruction and a normal bone scintigram. The abnormalities were differently shaped in the gamma camera images which indicates a possibility of differentiating between different extensions of neoplastic growth in the temporal bone. However inflammation as well as neoplastic processes influence on bone accumulation of  $^{99m}\text{Tc}$ -DP and abnormal accumulation patterns could have several explanations for instance neoplasia, inflammation or both (Greenberg et al., 1968; Galasko & Doyle 1977; Tofe et al 1975; Treves et al 1976; Bergstedt & Haverling, 1978; Bergstedt & Lind, 1978; Bergstedt & Lind 1979).

The abnormal accumulation in the mastoid region of patient I (Fig. 2) might depend on inflammatory reactions provoked by earlier surgical excision of the auricle and the cartilaginous part of the external meatus or by the small biopsies taken later from this area. However biopsy demonstrated a recurrence of the tumour growth and the mastoid region and the bony part of the external meatus had been left intact by the surgeon. It is therefore more likely that the abnormal accumulation was caused by a spread of malignant growth into the cortex of the mastoid process which also explained the destruction demonstrated by tomography.

The abnormal accumulation in the mastoid region of patient II was most probably explained by the tumour recurrence verified by histopathological examination which had changed the original diagnosis from basal cell to squamous cell carcinoma. However it cannot be excluded even if not probable that the pathologic uptake of isotope could be explained by the excision of perist of the region in 1977 and therefore this case also illustrates the necessity of careful analysis of scintigraphic findings in correlation to patient history in order to lessen the risk of false-positive diagnosis of neoplastic engagement of bone tissue.

The engagement of the whole left pyramid of patient III indicates an influence on bone far beyond the radiologically demonstrable bone destruction of the tumour. This patient had a chronic otitis of the left ear and the abnormal accumulation of the isotope might in part be explained by the chronic inflammatory reactions. However no acute exacerbations had occurred during the last few years and so the abnormal accumulation was presumably explained by neoplastic influence on the bone. Despite the macroscopically radical excision of the tumour area the patient eventually died from local recurrence. The scintigraphy findings probably correlated better to tumour extension than did the tomography findings.

The basal cell cancer tissue of patient IV





Fig 7 Scintigram of patient V presented according to Fig 1 Pathologic accumulation in the entire right pyramidal region.

destruction could be seen. Histologic examination verified the diagnosis. The patient has been observed without signs of recurrence for 2½ years.

#### Patient V

A woman aged 59 with residual bilateral external otitis for several years. Because of a therapy resistant external otitis of the right ear since Dec 1978 she was finally referred to an otologist for treatment. The external meatus was compressed by tumour tissue growing within the meatus and infiltrating the parotid region. Histologic examination demonstrated a medium-high differentiated squamous cell carcinoma. Radiography and polytomography demonstrated destruction of the lateral parts of the bony meatus and the dorsal part of the

fossa of the temporomandibular joint. There were neither radiologic nor audologic signs of affection of the inner ear.

Scintigraphy was performed and there was an abnormally high accumulation of  $^{99m}\text{Tc}^{\text{MDP}}$  around the outer meatus in the temporal fossa and in the lateral petrous part of the temporal bone (Fig 7). The patient was treated with  $\gamma$  radiation (64 Gy) in fractionated doses. A second scintigraphy 2 weeks after the last irradiation dose demonstrated an increase in the pathologic accumulation of the isotope.

The patient was operated upon and the cancer was found to be growing around the external meatus and within the middle ear. Radical excision had not been achieved according to histologic examination and the patient was further treated with cytotoxic drugs.



Fig 8 Scintigram of patient VI presented according to

its extent as well as in evaluating the prognosis of cancer of the ear. They also illustrate the necessity of a careful analysis of patient's history, symptoms and clinical signs, in relation to results obtained by scintigraphic methods. This is necessary in order to relate different causes of increased accumulation of  $^{99m}\text{Tc}$ -DP, such as chronic or acute inflammation, earlier surgery, neoplastic growth, etc.

It is not possible at the present level of experience to assess completely bone scintigraphy as a method for diagnosing malignancies of the ear in comparison with other methods. Further information concerning the  $^{99m}\text{Tc}$ -DP distribution in the skull bones of normal individuals as well as of patients suffering from different malignant, inflammatory and other diseases must be investigated in order to prepare the necessary basis for adequate interpretation of scintigram results.

## ZUSAMMENFASSUNG

Der potentielle Wert der Knochenzintiografie für Diagnostik und Prognosebestimmung des Ohrkarzinoms ist hervorgehoben und illustriert, mit Resultaten von 6 Patienten mit hochdifferenzierten Ohrkarzinomen.  $^{99m}\text{Tc}$ -Diphosphonat und spezielle Otorhinolaryngologische Untersuchungen wurden angegeben.

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extended along the bony walls of the external meatus but no bone destruction could be demonstrated by tomography or at operation. This patient is the only one without demonstrable bone destruction and accordingly this patient had a normal accumulation of  $^{99}\text{Tc}^{\text{m}}\text{DP}$ . He is alive and without signs of recurrence at 2½ years after operation.

Patient V had an extensive growth of a squamous cell carcinoma causing bone destruction demonstrable by polytomography in the lateral part of the temporal bone region and a pathologically high accumulation of  $^{99}\text{Tc}^{\text{m}}\text{DP}$  in the corresponding region. The tumour growth extended beyond the radiologically demonstrable destruction and probably corresponded better to the wider extension of the abnormally high accumulation of  $^{99}\text{Tc}^{\text{m}}\text{DP}$ .

Patient VI also had an extensive growth of squamous cell carcinoma causing radiologically demonstrable destruction and at operation the tumour growth was found to extend far beyond the limits of demonstrable bone destruction. The tumour extension seemed to correspond better to the region of abnormal  $^{99}\text{Tc}^{\text{m}}\text{DP}$  accumulation than to the region of radiologically demonstrable destruction.

All patients but no IV had squamous cell carcinomas and pathologic accumulation of  $^{99}\text{Tc}^{\text{m}}\text{DP}$  in the region affected. Furthermore none of the patients but no IV could be surgically treated with radical excision of the tumour without recurrence despite excision margins far beyond the margins of radiologically visible destruction. In contrast patient IV had a basal cell carcinoma without radiologically demonstrable destruction or abnormal accumulation of  $^{99}\text{Tc}^{\text{m}}\text{DP}$  in the region affected. This patient is the only one with surgically radical excision still alive without recurrence 2½ years after treatment.

These findings indicate the prognostic value of temporal bone scintigraphy in combination with radiological examination of patients harbouring carcinomas of the ear. The extension of the region of pathologic accumulation of  $^{99}\text{Tc}^{\text{m}}\text{DP}$  was in all cases greater than cor-

responding regions of radiologically demonstrable destruction. The findings at surgery and poor prognosis with recurrences in all patients but case IV also indicate that the larger regions of tumour involvement demonstrated by scintigraphic findings should be more relevant for therapeutic and prognostic assessment than the smaller region of destruction demonstrated by radiography. However it must be borne in mind that the extension of a hot spot is for physical and technical reasons somewhat larger in the gamma camera image than in reality.

The scintigraphic oblique lateral projection, corresponding to the Stenver projection of the ear, was found to be of value in examining the pyramid region especially in patient III. It is seen from Figs 4 and 5 that this projection demonstrated the accumulation and the abnormal side differences more clearly than did the A/P and the lateral projections. Furthermore the oblique lateral projection in connection with the lateral projection creates different topographic views of the bone structure and alterations in the orientation of structures in the two projections might give further information on the site and extension of the affected bone.

All scintigrams obtained after radiotherapy of the cancerous ears (patients I, II, III and V) showed an increase in the abnormal accumulation in the particular area in comparison with those scintigrams obtained before radiotherapy. This change in bone engagement is difficult to evaluate at present. The accumulation of  $^{99}\text{Tc}^{\text{m}}\text{DP}$  in normal and tumorous bone is reported not to be influenced by radiotherapy per se (Cox 1974; Gates & Gons, 1976; Lind & Nathanson 1977; Bergstedt & Lind 1979). Thus changes in  $^{99}\text{Tc}^{\text{m}}\text{DP}$  accumulation might offer information on the progression or regression of malignant growth or inflammatory reactions following irradiation.

The results presented indicate the potential value of bone scintigraphy in demonstrating the tumorous influence on the bone tissue.

## 1 Patients with prolonged bleeding time

|     | Age<br>(years) | Ivy bleed<br>ing time<br>(minutes) | Cause of the prolonged bleeding | Treatment                   |
|-----|----------------|------------------------------------|---------------------------------|-----------------------------|
| S   | 13             | 19                                 | Thrombasthenia                  | AMCA + fresh<br>whole blood |
| R   | 22             | 18                                 | No definite diagnosis           | AMCA                        |
| I   | 22             | 15                                 | Thrombasthenia                  | AMCA + fresh<br>whole blood |
| K.  | 47             | 14                                 | Thrombasthenia                  | AMCA                        |
| H   | 32             | 14                                 | Enhanced fibrinolytic activity  | AMCA                        |
| O   | 49             | 13                                 | Intake of acetyl-salicylic acid | 0                           |
| M   | 52             | 14                                 | Intake of acetyl-salicylic acid | 0                           |
| II  | 5              | 16                                 | Intake of acetyl-salicylic acid | 0                           |
| R.  | 39             | 18                                 | Intake of acetyl-salicylic acid | AMCA                        |
| M   | 40             | 15                                 | No definite diagnosis           | AMCA                        |
| J.D | 41             | 17                                 | Unknown                         | 0                           |
| E   | 6              | 18                                 | Intake of acetyl-salicylic acid | AMCA                        |
| L   | 69             | 16                                 | Unknown                         | 0                           |
| L   | 51             | 14                                 | Intake of acetyl-salicylic acid | AMCA                        |
| M   | 57             | 13                                 | Thrombasthenia                  | AMCA                        |
| V   | 8              | 18                                 | No definite diagnosis           | AMCA                        |
| P   | 33             | 18                                 | Intake of acetyl-salicylic acid | 0                           |
| L   | 8              | 14                                 | Unknown                         | 0                           |
| C   | 15             | 15                                 | No definite diagnosis           | AMCA                        |
| P   | 20             | 14                                 | No definite diagnosis           | AMCA                        |
| I.M | 9              | 18                                 | Intake of acetyl-salicylic acid | 0                           |
| N   | 55             | 18                                 | No definite diagnosis           | AMCA                        |
| S   | 38             | 14                                 | Intake of acetyl-salicylic acid | AMCA                        |
| O   | 5              | 18                                 | No definite diagnosis           | AMCA                        |
| I.L | 49             | 18                                 | Intake of acetyl-salicylic acid | 0                           |

platelet count was done according to Björk (1959) and Duke bleeding time as described by Nilsson (1974). Platelet adhesiveness according to Hellén's method (Hellén 0). Aggregation studies of the platelets with an aggregation method were performed in a Syton Dual Channel Aggregation Module as described by Cronberg (1970).

**Coagulation:** Coagulation time in glass test tubes and in plastic tubes, recalcification time, plasma and activated partial thromboplastin time, ATP (General Diagnostica) (Nilsson 1974) were studied. Factor VIII clotting activity (VIII C) was determined biologically as described by Nilsson et al. (1957). Factor VIII related antigen (VIII R Ag) immunochromatometrically in the way described by Holmberg & Nilsson (1973). Factor IX, one stage prothrombin time (P&P), factor V, fibrinogen, factor XIII (fibrin stabilizing factor) were determined by methods reported earlier (Nilsson 1974, Jernhag et al. 1979).

**Fibrinolysis** was tested by determination of fibrinolytic activity of plasma and resuspended euglobulin precipitate on fibrin plates, euglobulin clot lysis time and fibrin degradation products (FDP) (Nilsson 1974, Nilsson & Olsson 1962).

The first Ivy test was performed on the day before operation. If the bleeding time was prolonged surgery was usually postponed for 2-3 weeks during which time a re-test and further investigation were performed.

## RESULTS

In 25 patients (8.3%) we found a prolonged bleeding time (Table 1). In 10 cases the condition could be related to intake of acetyl-salicylic acid since the bleeding time at re-testing after withdrawal of the drug was normal. In 3 more cases with no history of acetyl-salicylic acid intake the bleeding on repeated assay also was normalized. In the remaining

## PREOPERATIVE TEST OF BLEEDING TIME IN EAR SURGERY\*

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(Received June 16, 1979)

**Abstract** In 300 patients subjected to ear surgery the bleeding time according to Ivy was determined preoperatively. In 25 cases prolonged bleeding time was found. Further investigations revealed mild thromboasthenia in 4 cases and enhanced fibrinolytic activity in one instance. In 10 patients the cause was intake of drugs containing acetylsalicylic acid. During operation the patients were treated with AMCA and in two cases fresh blood also was given. Even slightly enhanced bleeding can affect the results in microsurgery of the ear. We therefore suggest that bleeding time should be determined as a preoperative routine in order to detect the patients in need of specific treatment. Patients also should be recommended to avoid drugs containing acetylsalicylic acid for a fortnight before surgery.

Good control of the bleeding in the operating field is a pre-requisite nowadays for successful microsurgery of the ear. The usual ways to achieve this condition are careful local hemostasis, various forms of low pressure anesthesia and locally administered adrenalin. In spite of these precautions some patients present bleeding problems. In order to find this group preoperatively we sought a screening procedure for the detection of disorders of hemostasis, e.g., conditions with defective platelet function, decreased factor VIII related antigen levels and high fibrinolytic activity. The test of choice seemed to be determination of the bleeding time *ad modum* Ivy. The aim of this investigation was three-fold, viz. (1) to detect patients with prolonged bleeding time preoperatively, (2) to elucidate the actual disorder, and (3) to find the specific treatment during surgery.

### MATERIAL

The material consisted of two series of altogether 300 patients operated on for various

diseases in the middle ear and air cell system. The first 100 patients were subjected to operative procedures which were planned to exceed 2 hours, e.g., mainly cholesteatoma surgery and reconstructive surgery in radical mastoidectomy cavities. In the second series, comprising 200 cases, all patients subjected to ear surgery were consecutively investigated. This means a predominance of stapedectomies and tympanoplastic procedures.

Since the results in the two groups did not differ, they have been combined. There were 140 women and 160 males aged between 3 and 79 (mean age 43) years. Patients with known bleeding disorders, malignancies, liver or renal diseases were excluded from the material.

### METHODS

The bleeding time was determined *ad modum* Ivy with the modification described by Borchgrevink and Waaler (Borchgrevink & Waaler 1958; Nilsson et al. 1963).

A blood pressure cuff on the arm was inflated to 40 mmHg pressure and three transverse cuts were made on the forearm. With a special knife (Gillette surgical blade E) three cuts were made 1 mm deep and 10-14 mm long. Mean bleeding time for the cuts was then determined. The normal value with this method is 6-12 minutes. In patients with prolonged bleeding time further platelet and coagulation and fibrinolytic studies were performed.

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achieved satisfactory control of the bleeding in all operations. The Ivy test is probably the most sensitive screening method for detecting conditions with prolonged bleeding time. In our study the cause could not be revealed in 10 patients and we cannot exclude a false positive test in 3 cases since the re-test gave a normal value.

In spite of its simplicity the Ivy test requires experienced laboratory personnel. Recently however General Diagnostics has introduced a device Simplate IIT<sup>TM</sup> which gives standardized cuts. A comparison between the results found with Simplate IIT<sup>TM</sup> and the method used in our study shows good agreement (Nilsson L. et al. in press). This means that Ivy's test could be performed in a satisfactory way in all surgery departments.

To ensure a good result in microsurgery of the ear this study thus clearly indicates that the bleeding time should always be determined as a preoperative routine in order to detect those patients who need specific treatment during operation.

## ZUSAMMENFASSUNG

Vor mikrochirurgischen Eingriffen am Mittelohr wurde bei 300 Patienten das Blutungszeit nach Ivy bestimmt. In 25 Fällen wurde eine Verlängerung der Blutungszeit festgestellt. Fernere Untersuchungen ergaben, vier Fällen eine milde Thromboesthenie und in einem Fall eine Erhöhung der fibrinolytischen Aktivität. Bei zehn Patienten bestand die Ursache in der Einnahme von azetylsalicylsäurehaltigen Medikamenten. Während der Operation wurden die Patienten mit Zyklopropanol (AMCA) behandelt und in zwei Fällen wurde Frischblut erbracht.

Auch eine geringfügige Senkung der Blutungszeit durch das Resultat mikrochirurgischer Eingriff am Ohr nachteilig beeinflussen. Es wird deshalb vorgeschlagen, als präoperativ Routine die Blutungszeit zu bestimmen und die Patienten anzufragen zu machen, bei denen eine besondere Behandlung angebracht ist. Außerdem sollte den Patienten die Einnahme von azetylsalicylsäurehaltigen Präparaten während zwei Wochen vor der Operation abgesehen werden.

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| Aggregation in Born aggrego- | Comments                       |
|------------------------------|--------------------------------|
| Pathol.                      | Thromboesthenia                |
| Pathol.                      | Thromboesthenia                |
| Pathol.                      | Thromboesthenia                |
|                              | Thromboesthenia                |
|                              | Enhanced fibrinolytic activity |
| Normal                       | No definite diagnosis          |
| Normal                       | No definite diagnosis          |
|                              | No definite diagnosis          |
|                              | No definite diagnosis          |
|                              | No definite diagnosis          |
| Normal                       | No definite diagnosis          |

effect of acetylsalicylic acid is longstanding. Even small amounts can give a prolonged bleeding for up to 2 weeks (Cronberg 1968). Obviously this substance is a component of my drugs of which the patients do not know the contents. In Sweden for instance we can find acetylsalicylic acid in about 60 registered drugs. We now cope with this problem by suggesting drugs containing paracetamol if the patients have any need for analgetics during the waiting time before operation.

During the operation the patients received AMCA, which is an inhibitor of fibrinolysis, also inhibits the local fibrinolysis thereby preventing the dissolution of haemostatic thrombi (Nilsson 1975). As an adverse effect we noted a transient nausea in some patients, specially when the drug was given by mouth. Vomiting may be detrimental to the result in reconstructive ear surgery. The prophylactic use of AMCA should be restricted to patients with verified prolonged bleeding time. In all the patients with demonstrated thromboesthenia fresh whole blood or platelet concentrates was at hand during the operation and had also to be given in some cases.

With the above-mentioned procedures we

Table II Coagulation and fibrinolytic data

| Case no       | APT time (seconds) | Ivy (minutes) | Platelets (10 <sup>9</sup> /l) | Platelet adh. Hellem (%) | AHF (factor VIII)    |                            |         | Fibrinolytic activity on fibrin plates |   |
|---------------|--------------------|---------------|--------------------------------|--------------------------|----------------------|----------------------------|---------|--|---|
|               |                    |               |                                |                          | Biolog. (VIII C) (%) | Immunochem (VIII R Ag) (%) | P&P (%) | Citrated plasma (mm <sup>2</sup> )     | Resusp. embol. prec. (mm <sup>2</sup> ) |
| 1             | 35                 | 19 17         | 796                            | 10                       | 110                  | 110                        | 109     | 0                                      | 81                                      |
| 3             | 33                 | 15            | 165                            | 8                        | 115                  | 118                        | —       | 0                                      | 85                                      |
| 4             | 38                 | 14 15         | 167                            | 13 1                     | 73                   | 69                         | 11      | 0                                      | 86                                      |
| 15            | —                  | 13            | 790                            | 4                        | —                    | —                          | —       | —                                      | —                                       |
| 5             | 35                 | 14 15         | 181                            | 12 1                     | 100                  | 130                        | 108     | 0                                      | 136                                     |
|               | 34                 | 13 18         | 1 6, 143                       | 10 6                     | 130                  | 139                        | 157     | 0                                      | 78                                      |
| 10            | 35                 | 15 15         | 51                             | 6                        | 705                  | 138                        | 170     | 0                                      | 51                                      |
| 16            | —                  | 16            | norm                           | norm                     | —                    | —                          | —       | —                                      | —                                       |
| 19            | —                  | 15            | 30                             | 70                       | —                    | —                          | —       | —                                      | —                                       |
| 20            | 39                 | 14 13         | 39 179                         | 10 4                     | —                    | —                          | —       | —                                      | —                                       |
| 22            | 35                 | 18 14         | 66 198                         | 14 77                    | 130                  | 16                         | 110     | 0                                      | 81                                      |
| 4             | 35                 | 18 >20        | 300 244                        | 30 6 1                   | 143                  | 128                        | 75      | 76                                     | 185                                     |
| Normal values | <45                | 6-1           | 1.5-340                        | 17-33                    | 60-160               | 60-175                     | 80-170  | 4±35                                   | 98-54                                   |

12 patients the further investigation revealed mild thrombasthenia in 4 cases and a condition with enhanced fibrinolytic activity in one patient. No definite diagnosis could be established in 7 cases (Table II).

### Treatment

Sixteen of the patients received AMCA (trans aminomethyl cyclohexane carbonic acid trans-examic acid Cyklokapron®). We used a recommended dose (Andersson et al 1965) of 0.01 g/kg body weight intravenously immediately preoperatively. This dose was repeated once or twice on the day of operation. In several cases AMCA was also given orally during the postoperative week with a dosage of 1.5 g four times daily. Fresh whole blood was available in all the patients with thrombasthenia and had also to be given on two occasions.

### DISCUSSION

The high incidence of prolonged bleeding time in our study was unexpected.

In 4 patients (1.3%) we could demonstrate mild thrombasthenia. These patients had a

normal number of platelets but prolonged bleeding time and decreased adhesiveness (Cronberg 1968). In 4 more patients who were placed in the group labelled 'no definite diagnosis' because of different or inconsistent findings on repeated investigations mild thrombasthenia could not be excluded. While the incidence of the more serious congenital disorders is relatively well known (Nilsson 1976) we have no clear opinion of the frequency of mild thrombasthenia. Some observations (Blombäck 1972) however indicate that it might be a common condition.

As expected several patients had a prolonged bleeding time due to intake of acetylsalicylic acid (Mielke et al 1969). This inhibits the release reaction of platelets and consequently the second wave of platelet aggregation as measured with Born's aggregometer (O'Brien 1968). In our material 10 patients (4%) had taken aspirin or related drugs. This figure should probably have been higher since in the last series we instructed our patients not to take this sort of medicine preoperatively. Still it could be proved that intake of acetylsalicylic acid was the cause in several cases. This could be explained by two facts. Firstly

## ATTIC RETRACTIONS FOLLOWING SECRETORY OTITIS

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(Received May 30, 1979)

**Abstract** At re-examination of 577 ears with secretory otitis 5 to 8 years after tubulation otomicrosurgery and tympanometry revealed different types of retractions of Shrapnell's membrane in 34% of them 4.2% had pronounced retraction with retraction of the ossicular anvil and 0.2% had atrophic cholesteatoma. There was close correlation between the frequency and severity of retraction on the one hand and the age of the patient at re-tubulation, the tympanometric conditions, and the pathology of pars tensa on the other. The continued progression is further promoted by lack of natural cleansing of the retraction. As the frequency of secretory otitis is very high in children the 4.2% of pronounced retractions constitute sufficient quantitative basis for direct progression to atrophic cholesteatoma.

The principal purpose of this study was to establish the character and frequency of pathological changes in the Shrapnell's membrane in well-treated secretory otitis. Another long-term object has been to investigate whether atrophic cholesteatoma is a direct after-effect of secretory otitis or whether it develops directly from retractions in Shrapnell's membrane.

### *Earlier investigations*

Several investigators have studied sequelae changes of pars tensa following secretory otitis (Stevens 1962, McKinnon 1971, Kilby et al 1972, Mawson & Fagan 1972, Kokko 1974, Bonding & Lorentzen, 1973, 1974, Tos & Poulsen, 1976) but to our knowledge there exists no systematic investigation of the changes in Shrapnell's membrane.

Mawson & Fagan (1977) found attic retractions in 2% of ears after well-treated secretory otitis. Kokko (1974) found attic cholesteatoma in 0.7% of ears and McKinnon (1971) in 1.0% when including both attic and sinus cholestea-

In our oldest material comprising patients with secretory otitis treated with tubulation before 1970 and re-examined in 1975 i.e. 5 to 8 years after treatment, we found changes of pars tensa in 55% of ears (Tos & Poulsen 1976) and cholesteatoma in 1%. In addition we found different degrees of retractions of pars tensa. Unfortunately this material was not systematically investigated with reference to changes in Shrapnell's membrane. Consequently in 1977 and 1978 we re-examined a new material of patients operated upon from 1971 to 1975 and after systematic investigations of the changes in Shrapnell's membrane these were divided into predetermined types of retraction.

## MATERIAL AND METHOD

The material comprises 577 ears from children under the age of 13, the majority being between 4 and 8 years of age with secretory otitis treated under general anaesthesia with adenotomy, myringotomy and exsuction of secretion and most frequently with tubulation.

The children were most often referred from an ENT specialist and our routine method has been to insert an Armatrone tube anteriorly or anterior inferiorly in the drum. In 58 ears tubulation was not performed but only myringotomy. Ten ears had much mucous secretion on this side but were not tubulated in deference to the wish of the parents. 7 ears had some serous secretion and 31 ears had small or negligible amounts of mucous secretion for which reason we estimated that tubulation was necessary. The patients were regularly



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Table II Relationship between age of patient at the re-evaluation and types of retraction of Shrapnell's membrane

| Age years | N of ears | Types of retraction |       |        |         |        | Cholesteatoma (%) |
|-----------|-----------|---------------------|-------|--------|---------|--------|-------------------|
|           |           | 0 (%)               | I (%) | II (%) | III (%) | IV (%) |                   |
| 5 and 6   | 40        | 90.0                | 5.0   | 5.0    | —       | —      | —                 |
| 7 and 8   | 120       | 79.2                | 5.8   | 11.7   | 1.7     | 0.8    | 0.8               |
| 9 and 10  | 130       | 67.7                | 13.1  | 19.2   | —       | —      | —                 |
| 11 and 12 | 137       | 60.6                | 18.2  | 15.3   | 5.1     | 0.7    | —                 |
| 13 and 14 | 57        | 50.9                | 17.5  | 28.1   | 3.5     | —      | —                 |
| 15 to 20  | 43        | 34.9                | 14.0  | 30.2   | 16.2    | 4.7    | —                 |
| Total     | 527       | 65.7                | 12.7  | 17.3   | 3.4     | 0.8    | 0.8               |

which could not be seen, in our case (0.2%) it extended to the antrum.

#### *Retractions and the duration and severity of the disease*

There was no relationship between the length of the observation period and the frequency of the different retraction types (Table I). The observation period is the time from the ejection of the last tubulus to the time of the re-examination. This is no measure of the duration of the disease since the duration before treatment is unknown. Furthermore children who were retubulated once or twice received treatment over a long period of time and accordingly the observation time was relatively short in most cases from 3 to 5 years. In the group with an observation period exceeding 5 years, only 16 ears had an observation period of 8 years.

There was a close correlation between the

age of the patient at the time of the re-examination and the frequency of retractions (Table II). The percentage of ears with a normal Shrapnell's membrane gradually decreased with age from 90% at the age of 5 or 6 to 35% at the age of 15–20 years ( $\chi^2$ -test,  $p < 0.001$ ).

The number of retubulations may express the severity or duration of the disease. The frequency of retractions in Shrapnell's membrane was no larger in ears which were tubulated twice ( $p > 0.9$ ) than in those tubulated only once. On the other hand retractions were significantly more frequent in ears which were tubulated three times than in those tubulated twice ( $p < 0.01$ ) and especially more frequent than in those tubulated only once ( $p < 0.001$ ). The ears which were only treated with myringotomy were as mentioned the least affected and Shrapnell's membrane was normal in most cases.

Table III Relationship between number of tubulations and types of retraction of Shrapnell's membrane

| No. of tubulations | N of ears | Types of retraction |       |        |         |        | Cholesteatoma (%) |
|--------------------|-----------|---------------------|-------|--------|---------|--------|-------------------|
|                    |           | 0 (%)               | I (%) | II (%) | III (%) | IV (%) |                   |
| One                | 380       | 65.8                | 13.4  | 16.1   | 3.7     | 0.8    | 0.3               |
| Two                | 66        | 60.6                | 10.6  | 25.8   | 1.5     | —      | —                 |
| Three              | 23        | 30.4                | 26.1  | 34.8   | 8.7     | —      | —                 |
| Paracotitis        | 58        | 84.4                | 5.2   | 8.6    | 1.7     | —      | —                 |
| Total              | 527       | 65.7                | 12.7  | 17.3   | 3.4     | 0.8    | 0.2               |

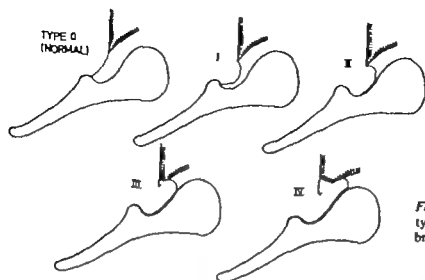


Fig 1 Schematic illustration of different types of retraction of Shrapnell's membrane

checked with audiometry and tympanometry 66 ears (12.5%) were retubulated once 23 ears (4.4%) twice. The patients were concluded approximately 1 year after ejection of the last tubulus.

For re-evaluation in the last 6 months of 1977 and the first 6 months of 1978 tone and speech audiometry tympanometry and otomicroscopy with a Zeiss operation microscope were performed. The degrees of Shrapnell's membrane retraction were divided into types from 0 to IV and the character and localization of all pars tensa changes were marked on a drawing of the drum.

## RESULTS

### Definition and frequency

Type 0 indicates a normal Shrapnell's membrane without retraction and with air between the neck of malleus and the Shrapnell's membrane (Fig 1). This occurred in 65.7% of the

ears (Table I). Type I indicates a retraction towards the neck of malleus but with air space, visible through Shrapnell's membrane (Fig 1). This type was found in 12.7% of the ears. Type II indicates that Shrapnell's membrane is retracted to the neck of malleus and that no air space was visible behind the membrane (Fig 1). This type occurred in 17.3% of the ears. Type III indicates that the retraction goes beyond the osseous annulus but the bottom of the retraction may be seen when the head is tilted. At the same time there may be a slight bone resorption corresponding to the osseous annulus (Fig 1). This type was found in 3.4% of the ears. Type IV indicates a distinct bone resorption of the osseous annulus and the retraction extends to the head of the malleus. However due to the bone resorption the bottom of the retraction may be seen (Fig 1). Atticus cholesteatoma was defined as a deep retraction pocket the bottom of

Table I Relationship between observation time and types of retraction of Shrapnell's membrane

| Observation time | No of ears | Types of retraction |       |        |         |        | Cholesteatoma |
|------------------|------------|---------------------|-------|--------|---------|--------|---------------|
|                  |            | 0 (%)               | I (%) | II (%) | III (%) | IV (%) |               |
| 3-5 years        | 335        | 65.4                | 12.8  | 17.6   | 3.3     | 0.6    |               |
| 5-8 years        | 197        | 66.1                | 12.5  | 16.7   | 3.6     | 1.0    |               |
|                  | 532        | 65.7                | 12.7  | 17.2   | 3.4     | 0.7    |               |

Table VI Relationship between pathology of pars tensa and types of retraction of Shrapnell's membrane

| Pars tensa | Types of retraction |                  |                   |                    |                  |                                  | Total<br>N=527<br>(%) |
|------------|---------------------|------------------|-------------------|--------------------|------------------|----------------------------------|-----------------------|
|            | 0<br>N=346<br>(%)   | I<br>N=67<br>(%) | II<br>N=91<br>(%) | III<br>N=18<br>(%) | IV<br>N=4<br>(%) | Chole-<br>steatoma<br>N=1<br>(%) |                       |
| Normal     | 57.5                | 26.9             | 26.4              | 5.6                | —                | —                                | 45.2                  |
| Abnormal   | 42.5                | 79.1             | 73.6              | 94.5               | 100.0            | 100.0                            | 54.8                  |

retraction of Shrapnell's membrane. Diffuse atrophy with retractions of pars tensa is characterized by a thin retracted ear drum with a diminished tympanic cavity but without adhesion to the promontory. Valsalva's test made the drum bulge forward prominently with Siegle's speculum. It was hypermobile. Retraction of Shrapnell's membrane occurred in 74% of these ears. In adhesive otitis the drum adhered to the promontory, was partly or entirely immobile and the middle ear was partly or entirely atelectatic. There were retractions of Shrapnell's membrane in all ears. Localized atrophy without retraction occurred either in the upper posterior or upper anterior quadrant or downward in tensa. Retraction of Shrapnell's membrane occurred in almost a third of these ears. Atrophy localized posteriorly with retraction onto the incudostape-

dial joint and a pex to the joint was accompanied in 88% of the ears by retraction of Shrapnell's membrane. Diffuse tympanosclerosis or fibrosis of the drum characterized a few ears with a considerably thickened drum, in the remaining ears there was circular or horse-shoe shaped tympanosclerosis in the entire circumference of the pars tensa. Localized tympanosclerosis either posteriorly anteriorly or downwards was in 35% of cases accompanied by retraction of Shrapnell's membrane. A combination of tympanosclerosis in one place and atrophy in another occurred frequently and Shrapnell's membrane was retracted in 66% of these ears.

Table VII shows the clear correlation between the severity of the pathology of pars tensa and the frequency of retractions of Shrapnell's membrane in the following order:

Table VII Relationship between different pathological conditions of pars tensa and types of retraction of Shrapnell's membrane

| Condition of pars tensa                | No. of<br>ears | Types of retraction |          |           |            |           | Chole-<br>steatoma<br>(%) |
|--|----------------|---------------------|----------|-----------|------------|-----------|---------------------------|
|  |                | 0<br>(%)            | I<br>(%) | II<br>(%) | III<br>(%) | IV<br>(%) |                           |
| Care atrophy                           | 3              | 69                  | 22       | 9         | —          | —         | —                         |
| Diffuse atrophy and retraction         | 11             | 26                  | 19       | 48        | —          | —         | —                         |
| Diffuse otitis                         | 13             | —                   | 23       | 38        | 38         | —         | —                         |
| Localized atrophy                      | 49             | 69                  | 14       | 16        | —          | —         | —                         |
| Localized atrophy and pex              | 17             | 1                   | 1        | 47        | —          | 0         | —                         |
| Diffuse tympanosclerosis               | 20             | 45                  | 33       | 15        | 18         | —         | —                         |
| Localized tympanosclerosis             | 79             | 65                  | 14       | 19        | 3          | 0         | —                         |
| Localized atrophy and tympanosclerosis | 48             | 44                  | 21       | 21        | 13         | 2         | —                         |
| Total                                  | 289            | 50.9                | 18.3     | 32.2      | 5.9        | 1.4       | 0.3                       |
| Normal pars tensa                      | 238            | 83.6                | 5.9      | 10.1      | 0.4        | —         | —                         |

Table IV Relationship between amount and character of the effusion and types of retraction of Shrapnell's membrane

| Effusion        | Types of retraction |                  |                   |                    |                  |                             | Total<br>N=577<br>(%) |
|-----------------|---------------------|------------------|-------------------|--------------------|------------------|-----------------------------|-----------------------|
|                 | 0<br>N=346<br>(%)   | I<br>N=67<br>(%) | II<br>N=91<br>(%) | III<br>N=18<br>(%) | IV<br>N=4<br>(%) | Cholesteatoma<br>N=1<br>(%) |                       |
| Impacted mucous | 71.7                | 79.1             | 74.7              | 72.2               | 100              | 100                         | 73.4                  |
| Little mucous   | 25.4                | 13.4             | 19.8              | 16.7               | -                | -                           | 2.4                   |
| Serous          | 9                   | 7.5              | 5.5               | 11.1               | -                | -                           | 4.2                   |

The vast majority of ears (95.8%) had mucous secretion at the first tubulation (Table IV). Among ears with copious mucous secretion there were no significant differences between the frequency of a normal Shrapnell's membrane and the different types of retraction ( $p > 0.1$ ). Among ears with small amounts of secretion there were more ears with a normal Shrapnell's membrane than ears with a type I retraction ( $p > 0.05$ ) though not more than types II or III (Table IV). Thus there is no correlation between the amount or kind of secretion on the one hand and the frequency of Shrapnell's membrane pathology on the other.

At the re-evaluation the middle ear pressures were divided into tympanogram types A, C<sub>1</sub>, C<sub>2</sub> and B (Table V) for practical reasons. Considerable middle ear effusion and type II was demonstrated in only 1.7% of the ears while 3% had type B without effusion; these had either adhesive otitis or a massive tym-

panosclerotic ear drum. A close correlation was found between the middle ear pressure and the retraction of Shrapnell's membrane. Among ears with a type A tympanogram there were significantly ( $p < 0.001$ ) more ears with a normal than with retracted Shrapnell's membrane of all types (Table V).

A significant relationship was found between pathology of pars tensa and retraction of Shrapnell's membrane (Table VI). In the group with a normal pars tensa there were more ears with a normal Shrapnell's membrane and fewer with retractions ( $p < 0.001$ ). More than 78% of ears with retraction of Shrapnell's membrane also had pathology of pars tensa.

The pathological changes of pars tensa were numerous (Table VII) and could be divided into atrophy, retraction and tympanosclerosis as well as combinations thereof. In diffuse atrophy the ear drum was thin but not retracted. Almost one third of these ears had

Table V Relationship between middle ear pressure at the re-evaluation and types of retraction of Shrapnell's membrane

| Tympanogram type   | Middle ear pressure (mmHgO) | Types of retraction |                  |                   |                    |                  | Cholesteatoma<br>N=1<br>(%) | Total<br>N=527<br>(%) |
|--------------------|-----------------------------|---------------------|------------------|-------------------|--------------------|------------------|-----------------------------|-----------------------|
|                    |                             | 0<br>N=346<br>(%)   | I<br>N=67<br>(%) | II<br>N=91<br>(%) | III<br>N=18<br>(%) | IV<br>N=4<br>(%) |                             |                       |
| A                  | 0 to -99                    | 81.7                | 50.7             | 45.1              | 22                 | 5.0              |                             | 68.9                  |
| C                  | -100 to -199                | 13.6                | 7.9              | 22.0              | 16.7               | 25.0             |                             | 16.9                  |
| C <sub>1</sub>     | -200 to -350                | 3.2                 | 11.9             | 25.3              | 44.4               |                  |                             | 9.5                   |
| C <sub>2</sub>     | Flat                        | 1.2                 | 3.0              | 4.3               | 16.7               | 50.0             | 100                         | 3.0                   |
| B without effusion | Flat                        | 0.3                 | 7.5              | 3.3               | -                  | -                |                             | 1.7                   |

Table VI Relationship between pathology of pars tensa and types of retraction of Shrapnell's membrane

| Pars tensa | Types of retraction |                  |                   |                    |                  | Chole-<br>steatoma<br>N=1<br>(%) | Total<br>N=57<br>(%) |
|------------|---------------------|------------------|-------------------|--------------------|------------------|----------------------------------|----------------------|
|            | 0<br>N=346<br>(%)   | I<br>N=67<br>(%) | II<br>N=91<br>(%) | III<br>N=18<br>(%) | IV<br>N=4<br>(%) |                                  |                      |
| Normal     | 57.5                | 26.9             | 26.4              | 3.6                | —                |                                  | 45.2                 |
| Abnormal   | 42.5                | 79.1             | 73.6              | 94.5               | 100.0            | 100.0                            | 54.8                 |

retraction of Shrapnell's membrane. Diffuse atrophy with retractions of pars tensa is characterized by a thin retracted ear drum with a diminished tympanic cavity but without adhesion to the promontory. Valsalva's test made the drum bulge forward prominently with Siegle's speculum it was hypermobile. Retraction of Shrapnell's membrane occurred in 74% of these ears. In adhesive otitis the drum adhered to the promontory was partly or entirely immobile and the middle ear was partly or entirely atelectatic. There were retractions of Shrapnell's membrane in all ears. Localized atrophy without retraction occurred either in the upper posterior or upper anterior quadrant or downward in tensa. Retraction of Shrapnell's membrane occurred in almost one third of these ears. Atrophy localized posteriorly with retraction onto the incudostape-

dial joint and a pexi to the joint was accompanied in 88% of the ears by retraction of Shrapnell's membrane. Diffuse tympanosclerosis or fibrosis of the drum characterized a few ears with a considerably thickened drum. In the remaining ears there was circular or horse shoe shaped tympanosclerosis in the entire circumference of the pars tensa. Localized tympanosclerosis either posteriorly anteriorly or downwards was in 35% of cases accompanied by retraction of Shrapnell's membrane. A combination of tympanosclerosis in one place and atrophy in another occurred frequently and Shrapnell's membrane was retracted in 66% of these ears.

Table VII shows the clear correlation between the severity of the pathology of pars tensa and the frequency of retractions of Shrapnell's membrane in the following order:

Table VII Relationship between different pathological conditions of pars tensa and types of retraction of Shrapnell's membrane

| Condition of pars tensa        | No. of ears | Types of retraction |          |           |            |           | Chole-<br>steatoma<br>(%) |
|--------------------------------|-------------|---------------------|----------|-----------|------------|-----------|---------------------------|
|                                |             | 0<br>(%)            | I<br>(%) | II<br>(%) | III<br>(%) | IV<br>(%) |                           |
| Diffuse atrophy                | 32          | 69                  | 22       | 9         |            |           |                           |
| Diffuse atrophy and retraction | 31          | 26                  | 19       | 48        | 3          |           |                           |
| Adhesive otitis                | 13          |                     | 23       | 38        |            |           |                           |
| Localized atrophy              | 49          | 69                  | 14       | 16        | 38         |           | 3                         |
| Localized atrophy and pexi     | 17          | 12                  | 12       | 47        |            | 0         |                           |
| Diffuse tympanosclerosis       | 20          | 45                  | 33       | 15        | 18         | 12        |                           |
| Localized tympanosclerosis     | 79          | 65                  | 14       | 19        | 3          | 5         |                           |
| Atrophy and tympanosclerosis   | 48          | 44                  | 1        | 21        | 13         | 0         |                           |
| Total pathology                | 289         | 30.9                | 18.3     | 23.2      | 3.9        | 1.4       |                           |
| Normal pars tensa              | 238         | 83.6                | 3.9      | 10.1      | 0.4        |           | 0.3                       |

Table IV Relationship between amount and character of the effusion and types of retraction of Shrapnell's membrane

| Effusion        | Types of retraction |                  |                   |                    |                  |                             | Total<br>N=577<br>(%) |
|-----------------|---------------------|------------------|-------------------|--------------------|------------------|-----------------------------|-----------------------|
|                 | 0<br>N=346<br>(%)   | I<br>N=67<br>(%) | II<br>N=91<br>(%) | III<br>N=18<br>(%) | IV<br>N=4<br>(%) | Cholesteatoma<br>N=1<br>(%) |                       |
| Impacted mucous | 71.7                | 79.1             | 74.7              | 72.2               | 100              | 100                         | 73.4                  |
| Little mucous   | 25.4                | 13.4             | 19.8              | 16.7               | -                | -                           | 22.4                  |
| Serous          | 9                   | 7.5              | 5.5               | 11.1               | -                | -                           | 4                     |

The vast majority of ears (95.8%) had mucous secretion at the first tubulation (Table IV). Among ears with copious mucous secretion there were no significant differences between the frequency of a normal Shrapnell's membrane and the different types of retraction ( $p > 0.1$ ). Among ears with small amounts of secretion there were more ears with a normal Shrapnell's membrane than ears with a type I retraction ( $p > 0.05$ ) though not more than types II or III (Table IV). Thus there is no correlation between the amount or kind of secretion on the one hand and the frequency of Shrapnell's membrane pathology on the other.

At the re-evaluation the middle ear pressures were divided into tympanogram types A, C<sub>1</sub>, C<sub>2</sub> and B (Table V) for practical reasons. Considerable middle ear effusion and type B was demonstrated in only 1.7% of the ears while 3% had type B without effusion; these had either adhesive otitis or a massive tym-

panosclerotic ear drum. A close correlation was found between the middle ear pressure and the retraction of Shrapnell's membrane. Among ears with a type A tympanogram there were significantly ( $p < 0.001$ ) more ears with a normal than with retracted Shrapnell's membrane of all types (Table V).

A significant relationship was found between pathology of pars tensa and retraction of Shrapnell's membrane (Table VI). In the group with a normal pars tensa there were more ears with a normal Shrapnell's membrane and fewer with retractions ( $p < 0.001$ ). More than 78% of ears with retraction of Shrapnell's membrane also had pathology of pars tensa.

The pathological changes of pars tensa were numerous (Table VII) and could be divided into atrophy, retraction and tympanosclerosis as well as combinations thereof. In diffuse atrophy the ear drum was thin but not retracted. Almost one third of these ears had

Table V Relationship between middle ear pressure at the re-evaluation and types of retraction of Shrapnell's membrane

| Tympanogram type   | Middle ear pressure (mmH <sub>2</sub> O) | Types of retraction |                  |                   |                    |                  | Cholesteatoma<br>N=1<br>(%) | Total<br>N=527<br>(%) |
|--------------------|--|---------------------|------------------|-------------------|--------------------|------------------|-----------------------------|-----------------------|
|                    |  | 0<br>N=346<br>(%)   | I<br>N=67<br>(%) | II<br>N=91<br>(%) | III<br>N=18<br>(%) | IV<br>N=4<br>(%) |                             |                       |
|                    | 0 to -99                                 | 81.7                | 50.7             | 45.1              | 22.2               | 50.0             |                             | 68.9                  |
| A                  | -100 to -199                             | 13.6                | 26.9             | 22.0              | 16.7               | 50.0             |                             | 16.9                  |
| C                  | -200 to -350                             | 3                   | 11.9             | 5.3               | 44.4               |                  |                             | 9.5                   |
| C <sub>1</sub>     | Flat                                     | 1.2                 | 3.0              | 4.3               | 16.7               | 50.0             | 100                         | 1.0                   |
| B without effusion | Flat                                     | 0.3                 | 7.5              | 3.3               | -                  | -                |                             | 1.7                   |
| B with effusion    |  |                     |                  |                   |                    |                  |                             |                       |

Table VI Relationship between pathology of pars tensa and types of retraction of Shrapnell's membrane

|                         | Types of retraction |                  |                   |                    |                  |                                  | Total<br>N=527<br>(%) |
|-------------------------|---------------------|------------------|-------------------|--------------------|------------------|----------------------------------|-----------------------|
|                         | 0<br>N=346<br>(%)   | I<br>N=67<br>(%) | II<br>N=91<br>(%) | III<br>N=18<br>(%) | IV<br>N=4<br>(%) | Chole-<br>steatoma<br>N=1<br>(%) |                       |
| Pathology of pars tensa |                     |                  |                   |                    |                  |                                  |                       |
| Normal                  | 57.5                | 26.9             | 26.4              | 5.6                | —                |                                  | 45.2                  |
| Retracted               | 42.5                | 79.1             | 73.6              | 94.3               | 100.0            | 100.0                            | 54.8                  |

retraction of Shrapnell's membrane. Diffuse atrophy with retractions of pars tensa is characterized by a thin retracted ear drum with a diminished tympanic cavity but without adhesion to the promontory. Valsalva's test made the drum bulge forward prominently. With Siegel's speculum it was hypermobile. Retraction of Shrapnell's membrane occurred in 74% of these ears. In adhesive otitis the drum adhered to the promontory, was partly or entirely immobile and the middle ear was partly or entirely atelectatic. There were retractions of Shrapnell's membrane in all ears. Localized atrophy without retraction occurred either in the upper posterior or upper anterior quadrant or downward in tensa. Retraction of Shrapnell's membrane occurred in almost one-third of these ears. Atrophy localized posteriorly with retraction onto the incudostape-

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Table VII Relationship between different pathological conditions of pars tensa and types of retraction of Shrapnell's membrane

| Pathology of pars tensa        | Types of retraction |          |          |           |            |           | Chole-<br>steatoma<br>(%) |
|--------------------------------|---------------------|----------|----------|-----------|------------|-----------|---------------------------|
|                                | No. of<br>ears      | 0<br>(%) | I<br>(%) | II<br>(%) | III<br>(%) | IV<br>(%) |                           |
| Diffuse atrophy                | 32                  | 69       | 22       | 9         |            |           |                           |
| Diffuse atrophy and retraction | 31                  | 26       | 19       | 48        | 3          |           |                           |
| Adhesive otitis                | 13                  |          | 23       | 78        | 38         |           | 3                         |
| Localized atrophy              | 49                  | 69       | 14       | 16        |            |           |                           |
| Localized atrophy and pexi     | 1                   | 1        | 12       | 47        | 18         | 0         |                           |
| Diffuse tympanosclerosis       | 20                  | 45       | 35       | 15        |            | 12        |                           |
| Localized tympanosclerosis     | 79                  | 65       | 14       | 19        | 3          | 5         |                           |
| Atrophy and tympanosclerosis   | 48                  | 44       | 1        | 21        | 13         | 0         |                           |
| Total pathology                | 289                 | 50.9     | 18.3     | 23.2      | 5.9        | 1.4       | 0.3                       |
|                                | 38                  | 83.6     | 5.9      | 10.1      | 0.4        | —         |                           |



adhesive otitis atrophy posteriorly and pexi diffuse atrophy and retraction of the drum atrophy and tympanosclerosis diffuse and localized tympanosclerosis and finally diffuse and localized atrophy in which only retractions of types I and II were found

## DISCUSSION AND CONCLUSION

1 Although retractions of types I and II do not present any immediate risk for the patient it is conceivable that over the years some patients may have a progression to types III or IV especially in those with a persisting tubal dysfunction and a negative middle ear pressure (Table V). Although we were unable to demonstrate any progression in the course of the relative short observation period (Table I) a re-evaluation of the material in some years is absolutely necessary especially since well defined criteria now are available for comparison and since the majority of children at that time will be over 15 years of age. As shown in Table II there is a clear tendency to progression of the retraction Shrapnell's membrane when this is related to the age of the patient. Recent screening investigations of healthy children have shown that during the first 2 years of life 50% of all healthy children have had secretory otitis for at least 3 months (Tos & Poulsen 1979). Although a spontaneous normalization of 84% was found a large number of the remaining children will appear for treatment between the age of 4 and 7 years after having had the disease from the second or third year of life. In view of this the age of the patient may reflect to some extent the actual beginning of the disease.

2 The extremely high frequency of secretory otitis places in a broader quantitative perspective the 4.4% of ears which in this material had retractions of types III and IV as well as atticus cholesteatomas. In most children a slight attack of secretory otitis will clear spontaneously without having been acknowledged leaving scarcely any after effects. However because of the very large

overall frequency of secretory otitis this group represents a considerable number of children. A hospital material such as this collected over 4 years represents a small percentage of all children with secretory otitis and presumably those with symptoms such as hearing impairment or acute otitis. As shown in Table IV the amount of effusion did not determine the frequency of retraction nor did we find more retractions in children with many episodes of acute otitis before tubulation. Therefore it may be assumed that a considerable number of untreated children have the disease in the same degree of severity as in this material and probably the same frequency of retraction of Shrapnell's membrane. In a population of 350 000 inhabitants 1 100 primary operations for chronic otitis and its sequelae were performed including a total of 476 cholesteatomas during a period of 10 years. When considering that 152 of these (Tos 1979b) had atticus cholesteatomas then the number of ears with type III and IV retractions must by far exceed the number of atticus cholesteatomas. Thus there is a quantitative basis for the assumption that atticus cholesteatomas develop directly from the retractions.

3 Earlier we have divided secretory otitis into three stages: an initial stage in which the histological changes start, a secretory stage in which effusion from the metaplastic mucosa dominates and middle ear secretion accumulates and finally a degenerative stage in which secretion dwindles and earlier formed mucous glands degenerate (Tos & Bak-Pedersen 1972; Tos 1976). Several conditions indicate that retractions of the Shrapnell's membrane of types II to IV develop primarily during the degenerative stage i.e. in the course of the long normalization process of the middle ear mucosa (a) in a total of 9 screening investigations (Tos et al. 1979) of 1 year-old and 2 year-old children (Tos & Poulsen 1979) including otoscopy of more than 2 000 children in which also Shrapnell's membrane was systematically investigated we did not find one single ear with retraction of types II to IV.

although 7-14% of the ears at each screening had secretory otitis in the secretory stage and a type B tympanogram furthermore 11-20% had a type C<sub>1</sub> tympanogram. (b) At the first tubulation of this material at which time most ears were in the secretory stage with much secretion (Table IV) retraction of types II to IV was not described. However Shrapnell's membrane was not so systematically investigated as at the re-evaluation. Yet retraction of types III and IV is so conspicuous that they hardly can be overlooked at otomicroscopy under general anaesthesia. (c) In the secretory stage the mucosa is swollen and thickened and the middle ear is more or less filled with secretion. A reversible retraction in pars tensa, will be abolished. (d) As shown in Table II we did not find type III and IV retractions among 5- and 6-year-old children.

4 The pathogenesis in the progression of retraction may differ but the primary cause of an incipient retraction is probably a dysfunction of the Eustachian tube and the negative middle ear pressure always present in secretory otitis. When using air-equalization methods, the tubal function is found to be poor during tubulation and prior to extrusion of the tubulus in 90% of the ears (Renvall & Holmquist, 1974; Poulsen & Tos, 1977). The tubal function normalizes gradually so that the middle ear pressure is normal after 1-1½ years in 43-50% (Renvall, 1975; Tos & Poulsen, 1977) after 1-5 years in 61% and after 5-8 years in 68% of the ears (Tos & Poulsen, 1977). Table V shows that also in this material the middle ear pressure is reduced in more than 30% of the ears and that the majority of retractions especially the most severe ones are found among these ears. In type I retractions flaccidity of the Shrapnell's membrane dominates with the possibility of further retraction. In types II, III and IV the dominant finding is adhesion of Shrapnell's membrane to malleus and here the natural cleansing of the retraction pocket will influence the progression. Accumulation of detritus in the retraction causes

infection which will promote bone resorption of the osseous annulus. This we have observed in several ears with type III and IV retractions. Even if the middle ear pressure were to normalize a progression of the retraction might continue due to the lack of natural cleansing. The progression of types II and III will be favoured by a prolonged reduced middle ear pressure or when adhesions are formed between epitympanum and mesotympanum and when the ventilation of epitympanum and antrum is blocked.

5 There is a close correlation between the changes in pars tensa and Shrapnell's membrane (Table VII) and especially in ears in which the effect of a prolonged low pressure in pars tensa has dominated as in adhesive otitis in atrophy with retraction and pexi as well as in diffuse atrophy and retraction. The largest percentage of ears was found with a pathological Shrapnell's membrane which supports the above mentioned pathogenetic considerations.

## ZUSAMMENFASSUNG

Bei otoskroskopischer und tympanometrischer Nachuntersuchung der 327 Ohren mit sekretorischen Otitis war den 3-8 Jahre nach der Tubulation bei 34% der Ohren verschiedene Grade der Retraktionen der Pars Flaccida gefunden. Bei 4,2% der Ohren waren die Retraktionen schwer mit Resorption der knöchernen Annelas, und 0,4% hatten Cholesteatome im Attica. Es wurde eine deutliche Korrelation zwischen der Häufigkeit und dem Schweregrad der Retraktionen auf der einen Seite und zwischen Alter der Patienten bei der Nachuntersuchung, Tympanometrieverhältnissen und Pathologie der Pars Tensa auf der anderen Seite gefunden. Die Progression der Retraktion wird von gestörter Selbstreinigung der Retraktion befördert. Da die Häufigkeit der sekretorischen Otitis bei Kindern sehr hoch ist, bilden die 4,2% der gefundenen schweren Retraktionen eine genügende quantitative Basis für eine deutliche Progression der schweren Retraktionen zum Atticocholesteatom.

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## CERVICO-OCULAR REFLEX IN THE NORMAL ADULT

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**Abstract** (1) The cervico-ocular reflex (COR) in humans is measured while the subjects ( $n=10$ ) stood on a rotatable platform in a dark room with the head fixed by stationary biteplate. Eye movements were measured in response to active and passive rotations about vertical axis. (2) The COR gain (horizontal eye movement/amplitude of body rotation) was as great as 22% at low frequency of body rotation (0.025 Hz). With increasing frequency (e.g., at 0.4 Hz) the gain decreased to about 7%. (3) The phase angle of the eye movement ranged generally between  $-80$  and  $240$  degrees with an average response around  $180$  degrees. (4) During active rotation the COR response was smaller than the responses measured during passive body rotation. (5) The principal conclusion is that in normal adult humans the COR does not stabilize the image on the retina during passive or active body rotations. A theoretical function for the COR is presented and discussed.

Early this century Bárány (1906) reported that he could observe reflexive eye movements in rabbits by fixing the rabbit's head and rotating its trunk about various axes. He concluded that the reflex was tonic since the eyes remained in their deviated position as long as neck torsion was maintained. Békésy & Rüttgen (1935) were the first to report similar reflexive eye movements in man. They described how they produced compensatory eye movements in a 18-year-old male with inexcitable labyrinthitis by rotating the man's trunk while holding his head fixed. Bárány (1918) disclosed that he could produce similar eye movements in newborn and premature infants. These reflexive eye movements could only be observed during the first two days after birth after which time they became obscured by spontaneous voluntary eye movements.

The non-vestibular origin of this reflex was

recognized quite early but it was not until thirty years later that the receptor sites were convincingly shown. The strongest evidence (Blomond & deJong 1969; Hikosaka & Maeda, 1973; McCouch et al. 1951) suggests that the receptors involved with the cervico-ocular reflex (COR) reside in the intervertebral joints and ligaments of the first three cervical vertebrae.

Although cervically induced eye movements were commonly seen in experimental animals and could be observed in infants and in adults under certain pathological conditions they were never seen in the normal adult (DeKleyn & Stenvers 1941). After considerable debate over whether the reflex actually existed in normal adults its presence was finally demonstrated when researchers began testing subjects in the absence of visual feedback (Philipszoon 1962; Philipszoon & Bos 1963; Suzuki 1972; Takahashi & Oyster 1974). Visual feedback was commonly eliminated by placing the subject in a dark room or having him close his eyes.

Fixation of the head together with the removal of visual input allows isolation of the COR from the two other primary oculomotor reflexes—the vestibulo-ocular reflex (VOR) which responds to head acceleration (Melville Jones & Milsum, 1965) and the opto-kinetic reflex (OKR) which responds to retinal image slip (Takahashi & Oyster 1974). It has been presumed that the COR acts together with the VOR and OKR to stabilize images on the retina during active head movements (Dich-

gans et al 1973 Fredrickson et al 1966 Hikosaka & Maeda, 1973 Hughes 1971 Meiry 1971 Suzuki 1972) An example of this oculomotor interaction was noted when Dichgans et al (1973) showed in the monkey that the cervico-ocular response increased dramatically after bilateral labyrinthectomy. However in humans there have been only two investigations indicating measurements which could verify the presumption that the COR actually acts to stabilize the retinal image in association with the VOR. Both of these have reported the gain and phase characteristics of eye movements (response) with respect to body movements (stimulus).

Takemori & Suzuki (1971) reported a gain of approximately 8% for rotations at 0.25 Hz. Meiry (1971) reported a gain of 30% for frequencies below 0.1 Hz which then gradually decreased to around 8% for frequencies about 0.4 Hz. Meiry also observed that the phase of the eye movements would aid in image stabilization. Takemori & Suzuki claimed that contrary to common belief the COR response in the normal adult would not aid in image stabilization—at least at 0.25 Hz the only rotational frequency which they used.

To resolve these conflicting data concerning the COR effect on image stabilization three questions were posed which were to be answered by this study:

- (i) What effect does the frequency of neck torsion have on the magnitude and direction of COR response?
- (ii) What effect does the individual's state of arousal or focus of attention have on the COR response?
- (iii) Is there a difference between the COR response seen during active as compared with passive neck torsion?

## METHODS

Ten subjects (age range 21 to 32 years) 5 male and 5 female took part in the experiments. They were not unaware of the nature of the

protocol however they did not know the purpose of the experiments.

Differential d.c. recordings between two electrodes (Beckman Miniature Electrodes 650437) placed at the outer canthus of each eye yielded the average horizontal component of eye movement. The electro-oculographic (EOG) signal was calibrated while the subject looked in turn at a series of strategically placed light-emitting diodes. As a precaution against corneo-retinal potential changes the EOG signal was recalibrated before each trial.

### *Passive body movement*

To stimulate the cervico-ocular reflex (COR) in isolation from the optokinetic and vestibulo-ocular reflexes (VOR) the subject stood in the dark with his (or her) head held by means of a dental biteplate clamped to a frame which was rigidly suspended from the ceiling. By bracing on the plate the subject could stabilize his head and allow all rotation to take place at the neck.

Passive body rotations were performed while the subject stood on a hydraulically driven servo-controlled platform. The platform rotated the subject's body about a vertical axis at an amplitude of  $\pm 0.4$  radians at five selected frequencies—0.025, 0.05, 0.1, 0.2 and 0.4 Hz. Individual trials ranged in length from 50 seconds (20 cycles at 0.4 Hz) to 160 (4 cycles at 0.025 Hz). The position of the subject's shoulders and feet (platform) was measured to indicate the absolute amount of neck torsion. This was done to ensure that all rotation occurred at the neck and not in the trunk since it has been reported (Biemond & deJong 1969) that pelvic rotation produces nystagmus.

One of the following three instructions was given to the subject prior to each trial:

- (i) Fixate in the dark—The subject was asked to fixate on a central low intensity red light (subtending 0.5 degrees visual angle) and to continue looking at it until it went out. After the light was turned off the subject was left in total darkness and was to continue staring at the spot where the light had previously been.

(ii) Respond to the tone - The subject was required to listen for the pseudo-random appearance of a soft tone from a speaker located directly behind him. This speaker location was chosen to minimize eye movements due to auditory localization (Howard & Templeton 1966). As soon as the subject heard the tone he was to respond by releasing a thumb-activated button mounted in a handle which he held in his hand. This terminated the tone. This technique served both to arouse the subject and to indicate trends of alertness or drowsiness throughout the duration of the experiment (2-3 hours in a dark room).

(iii) Close eyes and relax - The subject was asked to gently close his eyes to relax, and to attempt to eliminate all thoughts from his mind (that is, not to daydream if possible).

#### *Active body movement*

To date all investigations into the COR have involved passive body rotations. There is evidence in other neurophysiological systems that responses to stimuli delivered during passive behaviors are non-existent or different from observations made in the presence of active behaviors (Evars & Tanji 1974; Melvill Jones & Watt, 1971). Therefore it is of interest to study the COR with active as well as passive body movements.

A turntable with a low moment of inertia and low frictional resistance was constructed. While standing on the turntable with his head fixed by a biteplate, the subject was capable of actively rotating his body using only his neck muscles. An attempt by the subject to rotate his head to the right resulted in a body rotation to the left since the subject's head was restrained while his body was free to rotate. A cyclic attempt by the subject to turn his head alternately to the right and left resulted in active body rotation similar to the passive rotations produced by the servo-driven platform. Through the use of the turntable it was therefore possible to produce active neck torsion without labyrinthine stimulation.

#### *Experimental procedure*

An experiment consisted of three to five trials involving active body rotation and fifteen trials of passive rotation. Each of the fifteen trials of passive rotation utilized one of the five stimulus frequencies and one of the three instructions stipulating mental activity. Before each experimental trial the EOG signal was calibrated and the subject was given an instruction to follow during the ensuing rotation. Rotation then began and lasted for one to three minutes depending on the frequency. During this time the subject was standing on the platform in total darkness and was restraining head movement by biting on the bite plate. Upon completion of rotation, the subject was allowed to sit and relax if he so chose before beginning the next trial.

#### *Data acquisition and analysis*

During each experimental trial several channels of data were recorded. These included: 1) the horizontal component of eye position; 2) the position of the servo-driven platform (during passive rotation); 3) the position of the turntable (during active rotation); 4) the position of the shoulders; 5) the state of the EOG calibration lights; and 6) the appearance of the audio signal together with the subject's response time. A six-channel pen recorder (Gould-Brush 260) was used to monitor the data while the experiments were in progress. The data were also recorded on an FM magnetic tape recorder (Honeywell 5600) for subsequent analysis using a PDP 11/50 computer (Digital Equipment Corporation).

Data from the passive experiments were reduced as follows. Using the platform signal as a reference (since it was a pure sinusoid) the computer divided each trial into cycles and then averaged the horizontal eye and shoulder position signals. The resulting cycle of averaged shoulder position was considered to be the stimulus and the concomitant cycle of averaged horizontal eye deviation was considered to be the response. The coefficients of

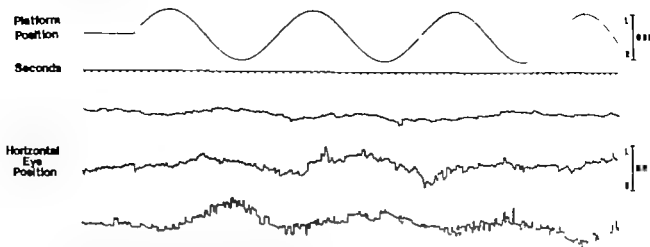


Fig 1 COR responses of 3 subjects illustrating their eye movements before and during body rotation. Note the varied character of the eye movements and that vertical

calibration for body rotation is twice that for eye movements ( $0.4 \text{ R} = 0.4 \text{ radians}$  (1 radian = 57 degrees)).

the first non-d.c. term i.e. the fundamental of the Fourier series describing these averaged signals were then found. These coefficients i.e. the amplitude and phase angle of the fun-

damental frequency component of each signal were used to calculate the gain and phase of the response. The gain of the COR response was found by dividing the amplitude of the output eye deviation by the amplitude of the input shoulder movement. The phase angle of the response was a measure of the difference in phase angle between the two fundamentals and was defined such that a phase angle of zero degrees would indicate an eye movement in the same direction as the stimulating body movement. A negative phase angle would signify that the eye response lagged behind the input shoulder motion.

A similar type of analysis was performed on the data collected during active body rotations; however, due to the fact that the rotation was actively produced, the frequency of rotation varied slightly from cycle to cycle. Therefore, only those sections of data exhibiting very small differences in frequency were averaged and used for further analysis.

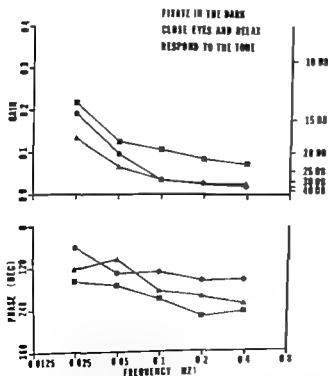


Fig 2 Mean COR response for 10 subjects for each of the three instructions given to the subjects. Upper: Gain vs frequency of neck torsion where gain is defined as the ratio of slow phase eye movement to amplitude of body rotation. Lower: Phase angle of response (in degrees) vs frequency of neck torsion. Gain may also be measured in decibels (dB) from vertical axis on right.

## RESULTS

### Form of cervico-ocular response

The protocol followed in the current study i.e. body rotation in the dark with the head held fixed with respect to the environment has demonstrated that cervically produced eye

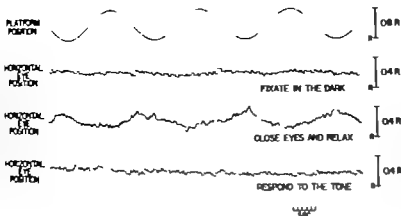


Fig. 3 Comparison between COR responses produced under three sets of instructions. Data shown are from one subject. Note that eye calibration (0.4 radians) is one-half that for the platform position.

movements have certain repeatable characteristics.

Fig. 1 contains a portion of the COR recorded from 3 different subjects. The data were selected to show the general character of neck induced eye movements. As illustrated in the figure during motionless periods (left side of figure) the subjects exhibited seemingly random eye movements around some central point. Upon the commencement of neck torsion, the baseline began to fluctuate. These fluctuations in the baseline will be referred to as slow phase eye movements (in opposition to the fast phase eye movements or saccades seen in nystagmus). Since typical values for gain and phase angle are given in the following section it is sufficient at this point to make two observations: first that the magnitude of the slow phase eye movements are significant (less than the magnitude of the neck movement) and second that the slow phase eye movement generally occurs in the direction opposite to the direction of platform (or shoulder) movement. That is a shoulder rotation to the right (clockwise rotation when observing the subject from above) results in a small eye rotation to the left (counter-clockwise when observing the subject from above).

As demonstrated in Fig. 1 slow phase eye movements appear to sum directly with spontaneous eye movements which occur in the absence of neck torsion. The random eye move-

ments which are present before rotation begins continue on throughout the period of neck rotation.

### *Effects of stimulus frequency*

At the lower frequencies of rotation employed cervically induced eye movements are most apparent. As the frequency of neck torsion is increased the magnitude of the resulting eye movement decreases until at 0.2 Hz there is little eye movement which can be correlated with the neck torsion stimulus.

The decline in response magnitude with increasing frequency is presented in Fig. 2. This figure contains the results of the computer analysis for all 10 subjects under each of the conditions used during the passive experiments. COR response gain is less than 3% at frequencies above 0.1 Hz for the Respond to the Tone and Fixate in the Dark instructions. The largest response is seen at the lowest frequency examined 0.025 Hz. At this frequency the maximum mean gain observed was approximately 22%.

The phase of the COR response generally ranged between  $-80^\circ$  to  $-240^\circ$  as seen in Fig. 2. There appears to be a slight increase in phase lag with increasing frequency of rotation although average phase angle response ranges around  $-180^\circ$ . The significance of this fact to ocular stability will be presented in the discussion.



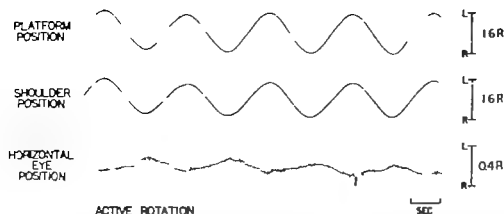


Fig 4 Active rotation in the dark with concomitant eye movements. Note that platform and shoulder positions are similar indicating that torsion is taking place at the neck rather than in the trunk region. Small high frequency oscillations which occur at peaks of eye movement record

are caused by electromyographic (EMG) activity sensed by the EOG electrodes. The maximum force and therefore EMG activity needed to provide active rotation is produced by neck muscles in the extremes of body position.

### Effects of subject arousal and focus of attention

The level of arousal and focus of attention of the subjects greatly affect the characteristics of the cervico-ocular reflex. Fig 3 shows typical

cal changes in the COR response of a single subject at a single frequency for the three instructions used in the experiments with passive rotations. The largest response for all frequencies was produced while the subjects were following the Close Eyes and Relax instruction. It is evident that the two instructions which required subject concentration ('Respond to the Tone' and 'Fixate in the Dark') resulted in responses of less magnitude than the instruction which allowed the subjects to relax (see Fig 2).

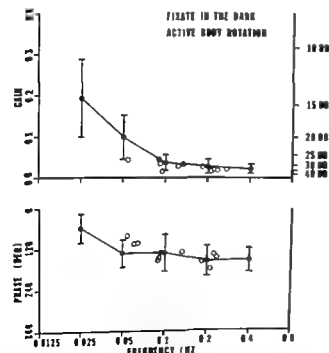


Fig 5 COR responses during active body rotation compared with mean COR response observed during passive body rotation under the instruction 'Fixate in the Dark'. Note that for passive rotation COR response is indicated by a mean and standard deviation at each frequency of rotation averaged for 10 subjects. Data points for active rotations are plotted individually.

### COR response during active rotation

Fig 4 shows a typical COR response during active body rotation. Approximately half of the subjects tested were able to produce smooth active body rotations while standing (with their head fixed) on the turntable. At the higher frequencies of rotation (around 0.4 Hz) the movements were near sinusoidal. When the subjects attempted to produce slower rotations the movements became more triangular and more irregular. Since the subjects were unable to produce smooth sinusoidal movements with frequencies below 0.05 Hz the frequency range examined during this part of the study was limited. The active movements analysed were between 0.05 and 0.4 Hz.

During preliminary experiments involving

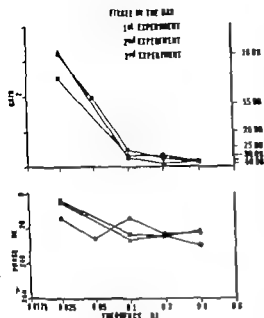


Fig. 6. COR response gain- and phase angle vs. frequency showing intra-subject variability of response. Data are from one subject tested on three different days.

active body rotation large voluntary eye movements were usually present. In an attempt to minimize these unwanted eye movements the subjects were asked to fixate on an imaginary point in space keeping their eyes centered and as motionless as possible while they rotated their bodies in the dark. This command ('Fixate in the Dark') was also used during the passive rotation experiments. The combined results of both the active and passive experiments under this command are shown in Fig. 5. The results indicate a variability between the COR response under active and passive conditions within the frequency range examined.

#### Inter- and intra-subject variability of response

In order to establish the repeatability of the data and to examine intra-subject variability in response repeated experiments were performed on 2 of the 10 subjects. Each of these subjects was tested on three occasions. The first and second tests were separated by ap-

proximately 3 months, the second and third by one week. Fig. 6 contains an example of the results obtained for the instruction 'Fixate in the Dark'.

Intersubject variability can be appreciated by referring to the standard deviation bars in Fig. 5.

## DISCUSSION

The most consistent characteristic of the cervico-ocular reflex response i.e. decrease in COR amplitude with increasing frequency of neck torsion confirms the general shape of the results reported by Meiry (1971) although he indicated a larger response amplitude over the entire frequency range than has been observed in this study. This could be due to the fact that he reconstructed the eye signal by removing saccades while these eye signal data are averaged. The amplitude discrepancy cannot be explained fully since Meiry did not describe certain important conditions of his experiments such as the subject's focus of attention and whether the figures represented an average or the largest responses.

Takemori & Suzuki (1971) used only one frequency of rotation (0.25 Hz) in their experiments but their experimental controls and method of data analysis were explained clearly. In one group of experiments their subjects underwent body rotation with their eyes closed. The observed gain of 8% (0.25 Hz) under these conditions is in agreement with the value found during the 'Close Eyes and Relax' command in the present study.

Also the results present the fact that neck induced eye movements almost always occur in a direction approximately opposite to the stimulating body movement. This is of utmost importance when considering the classical concept of the COR's role in image stabilization (see Ocular Stability and COR Function).

#### Subject level of arousal

Historically the importance of the subject's level of arousal in measurements of oculo-

motor function was realized because of contradictory data reported during vestibular testing. Collins & Guedry (1962) discovered that when their subjects were mentally active no decline in nystagmus was evident even during long periods of angular acceleration. However, during states of mental relaxation nystagmic response was much smaller and occasionally disappeared prior to the end of the stimulus.

Similarly Sokolovski (1966) noted that calorically induced nystagmus was consistently greater and more regular in form in subjects who were mentally active when compared with relaxed subjects. Therefore it is now generally agreed that drowsiness, boredom and fatigue suppress vestibular response while mental activity enhances it.

This is in direct contrast to the results found in this study for the cervico-ocular reflex. The instruction 'Close Eyes and Relax' required no concentration yet resulted in the largest response (Figs 2-3). The other two instructions required the subject to focus his attention. During the instruction 'Respond to the Tone' the subjects were repeatedly reminded of their task by the need to respond to each occurrence of the audible tone. This instruction produced the smallest COR responses (Fig. 2).

#### *Active rotation*

Most of what is known about the cervico-ocular reflex has been derived from passive neck torsion experiments. Because of the accumulating body of data on the inhibition or transformation of reflexes when measured during functional behaviors (Evars & Tanji 1974; Melvill Jones & Watt 1971) the question arose whether active neck rotation might not result in eye movements which differed from the response produced by passive neck torsion. The data do not conclusively answer this question since the behavior used was not functional; however, the movement stimuli were active rather than passive. To this extent then the results as presented in Fig. 5 show

the resemblance between COR data produced by either passive or active body rotation.

#### *Ocular stability and COR function*

It has been stated by many investigators that the COR functions to aid in image stabilization upon head movement. That is, when the head is rotated to the right, cervical receptors, stimulated by the resulting neck torsion, aid in initiating an eye movement to the left. The two opposing rotations cancel each other and the eye direction in space tends to remain unchanged, implying that retinal image slip is reduced by the COR. This stated function of the COR will now be reexamined in light of the results of the present study.

While the gain of the VOR commonly ranges between 40 and 60% (or as high as 95% under certain conditions (Barr et al. 1976)) the highest mean COR gain seen during this study was approximately 22% (although gains for individual runs on occasion ranged as high as 35% and gains for individual cycles of rotation ranged as high as 55%). COR response was found to be largest at very low frequencies of neck torsion—frequencies below those involved in normal head movement. If it is assumed that normal human head movements have a frequency content between 0.1 and 5.0 Hz (Melvill Jones & Milsum 1965) then one would expect the COR gain during normal daily movements to be between 7 and 10%. Therefore upon a normal head movement of 20° one might expect an eye movement of 7° or less due to cervical stimulation.

Consider now the direction of eye movement. The experimental responses for all test conditions exhibited a phase angle between -80° to -240°—the average response phase being close to -180°. An eye movement which would tend to compensate for a head movement would be in a direction to oppose the head movement—a phase angle of 180° between the eye and the head movements. However, in these experiments the head was fixed and neck torsion occurred as a result of body

otion. An assumption has been made here that neck receptors will be stimulated equally well by a body rotation (head fixed) as by a head rotation (body fixed). However the direction of rotation would be opposite. That is a head rotation to the right (body fixed) would be equivalent to a body rotation to the left (head fixed). Therefore an eye movement which would aid in eye stabilization (as does the VOR) would be in the same direction as the body movement—a phase angle of  $0^\circ$  between the eye and body movements. In general then, since eye movement and body movement were  $180^\circ$  out of phase rather than reducing retinal image slip the COR response enhanced it. A similar finding was reported by Gesty (1976) working with rabbits.

What then is the function of the cervico-ocular reflex. A speculative answer to this question is suggested as follows. The vestibulo-ocular reflex provides an image of head acceleration to the oculomotor centers to help in eye fixation during head movement. It is proposed that the COR provides an image of body movement to the oculomotor centers. The need for separate head image and body image to provide eye fixation becomes apparent during complex movements of head and body. For example assume that the head is moving to the left at  $5^\circ/\text{sec}$  and the body is also moving to the left but with a velocity of  $70^\circ/\text{sec}$ . In this case the VOR would produce an eye movement toward the right (responding to the  $5^\circ/\text{sec}$  head rate) the COR would sense the relative neck torsion to the left ( $15^\circ/\text{sec}$  rate) and would produce an eye movement also to the right. The combined eye movement produced by VOR and COR would therefore tend to reduce retinal image slip.

Despite the fact that the summation of VOR and COR effects will not always occur as it does in the example the concept of an eye movement opposed to head motion (VOR) and an eye movement opposed to body movement (COR) is satisfying. Although such a function for the COR is speculative it suggests the path

## ACKNOWLEDGMENTS

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## ZUSAMMENFASSUNG

1. Der cervico-okuläre Reflex (COR) beim Menschen wurde gemessen, während die Versuchspersonen (Zahl 10) auf einer Drehplattform standen. Der Kopf der Versuchsperson wurde von einer stationären Beißplatte festgehalten. Die Augenbewegungen wurden als Reaktion der aktiven und passiven Körperrotation um einer vertikalen Achse gemessen.

2. Der Verstärkungsfaktor des COR (d.h. horizontale Augenbewegung/Schwingungsbereich der Körperrotation) war so stark als 22% bei einer schwachen Frequenz der Körperrotation (0,025 Hz). Bei zunehmender Frequenz (z.B. 0,4 Hz) nahm der Verstärkungsfaktor bis zu 5% ab.

3. Der Phasenwinkel der Augenbewegung lag in den meisten Fällen zwischen  $-80^\circ$  und  $-40^\circ$  Grad mit einer durchschnittlichen Reaktion von ungefähr  $-180^\circ$  Grad.

4. Während der aktiven Rotation war der COR den Reaktionen der passiven Körperrotation ähnlich.

5. Das Hauptergebnis ist folgendes: Beim normalen wachen Menschen unterstützt nicht der COR die Stabilisierung von dem Ercheinungsbild auf der Netzhaut während der passiven oder aktiven Körperrotationen. Eine theoretische Funktion des COR wird vorgelegt und diskutiert.

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motor function was realized because of contradictory data reported during vestibular testing. Collins & Guedry (1962) discovered that when their subjects were mentally active no decline in nystagmus was evident even during long periods of angular acceleration. However during states of mental relaxation nystagmic response was much smaller and occasionally disappeared prior to the end of the stimulus.

Similarly Sokolovski (1966) noted that calorically induced nystagmus was consistently greater and more regular in form in subjects who were mentally active when compared with relaxed subjects. Therefore it is now generally agreed that drowsiness, boredom and fatigue suppress vestibular response while mental activity enhances it.

This is in direct contrast to the results found in this study for the cervico-ocular reflex. The instruction *Close Eyes and Relax* required no concentration yet resulted in the largest response (Figs 2, 3). The other two instructions required the subject to focus his attention. During the instruction *Respond to the Tone* the subjects were repeatedly reminded of their task by the need to respond to each occurrence of the audible tone. This instruction produced the smallest COR responses (Fig. 2).

#### *Active rotation*

Most of what is known about the cervico-ocular reflex has been derived from passive neck torsion experiments. Because of the accumulating body of data on the inhibition or transformation of reflexes when measured during functional behaviors (Evarts & Tanji 1974; Melvill Jones & Watt 1971) the question arose whether active neck rotation might not result in eye movements which differed from the response produced by passive neck torsion. The data do not conclusively answer this question since the behavior used was not functional; however the movement stimuli were active rather than passive. To this extent then the results as presented in Fig. 5 show

the resemblance between COR data produced by either passive or active body rotation.

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Consider now the direction of eye movement. The experimental responses for all test conditions exhibited a phase angle between -80° to -240°—the average response phase being close to -180°. An eye movement which would tend to compensate for a head movement would be in a direction to oppose the head movement—a phase angle of 180° between the eye and the head movements. However in these experiments the head was fixed and neck torsion occurred as a result of body

## VISUAL SUPPRESSION OF CALORIC NYSTAGMUS AND OPTOKINETIC RESPONSES IN CATS

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**Abstract:** Previously it was reported that loss of visual suppression (VS) was accompanied by disturbance of optokinetic nystagmus (OKN) in monkeys with lesions in the flocculus. In the present experiment with cats, OKN was investigated after inflicting lesions on either superior colliculus (SC) or inferior olive (IO), both of which are considered to be the main prefloccular relay nuclei to mediate visual signals to the flocculus. VS of caloric nystagmus was recognized in all the IO-lesioned cats and OKN remained normal except in one cat. After the left SC lesions, loss of VS was evidently revealed and optokinetic stimuli produced directional preponderance to the left with disorganized responses to the right in 7 of 9 cats. The present findings suggest that the SC may be an important relay nucleus to the flocculus in conveying visual signals responsible for VOR gain.

The flocculus receives primary vestibular signals (Brodal & Horvik 1964) as well as visual signals from the retina (Mackawa & Simpson 1973; Mackawa & Takeda 1975) and sends inhibitory outputs to second-order vestibular neurons which mediate vestibulo-ocular reflexes (VOR) in an excitatory and inhibitory way (Ito et al. 1973a, b). On the basis of the neuronal circuitry described above, the flocculus controls VOR gain (eye velocity/head velocity) by utilizing visual signals (Ito et al. 1974; Ghelarducci et al. 1975; Ito 1976; Lisberger & Fuchs 1978a, b). The gain control of the flocculus might be executed with the aid of oculomotor function which works to stabilize the retinal images while the head is moving. In fact, a failure in suppressing the slow-phase velocity of caloric nystagmus in man is well

correlated with disturbances of the oculomotor functions e.g. optokinetic response, smooth pursuit eye movements and ocular position of gaze (Sato et al. in press). Under such conditions as reduced or loss of gain control of VOR, optokinetic nystagmus (OKN) involving smooth pursuit eye movements might be also disturbed even in cats. The present experiment was concerned with the investigation of OKN in cats with lesions in the superior colliculus (SC) or the inferior olive (IO), both of which are thought to be the prefloccular relay nuclei to mediate visual signals to the flocculus (Mackawa & Simpson 1973; Mackawa & Takeda, 1975).

### METHODS

Forty-two cats were used in the present experiments. The operative procedures for the animals, the techniques of fixing the body of the awake cat, those of inducing and recording caloric nystagmus, and the method for calculation of visual suppression (VS) in slow-phase velocity were described previously (Kato et al., 1979a). Anesthesia, histological identification of electrolytic lesions and the experimental procedures for verifying the IO or the SC were also described in detail in the previous report (Kato et al. 1979b). Electrolytic lesions of the IO or the SC were focused only on the left side. After inflicting lesions on

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Table II Relationships between VS of caloric nystagmus and lesion site

| Lesion site | Loss of VS | VS |
|-------------|------------|----|
| rostral     | 6          | 4  |
| caudal      | 0          | 5  |
| SC          | 7          | 2  |

abnormality of OKN OKN-DP to the left alone falls in the lower right corner (shaded area) and OKN DP to the right alone in the upper left corner (shaded area). The other three areas signify bilateral OKN diminution (lower left square) and unidirectional diminution of OK responses combined with OKN DP. The lowest and highest nystagmus beats were 33 and 153 which are indicated by horizontal and vertical dotted lines respectively (Fig. 1). Thus the normal range fell in the hexagonal area. After the follow-up studies were completed the brain of the animal was perfused with 10% formal-saline solution through the left cardiac ventricle. The extent

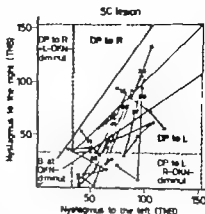


Fig. 3 Optokinogram using TNB as parameter OKN in cats with SC lesions tends to show OKN-DP to the left with diminished OK responses to the right except for 2 cats (14, 15) belonging to Group I.

of lesions was determined by serial sections of paraffin-embedded specimens and reproduced by a projecting device.

## RESULTS

Of 42 cats investigated 30 survived the electrolytic lesions of the IO or the SC. In 25 of the 30 cats, the control test could be performed thoroughly before and after lesions were made. In 7 of 25 cats the lesions were found outside the target area. The data for those 7 cats were therefore discarded ultimately. Table II summarizes the relationships between VS of caloric nystagmus and the lesion site. VS of caloric nystagmus was revealed in all the IO-lesioned cats. With the SC lesion VS was not recognized in 7 of 9 cats. OKN investigated on these cats is described according to lesion site.

### IO lesion

In 4 of 9 cats lesions of the IO were found to cover the rostral part, but not the caudal part of the medial accessory olive (MAO) and in the remaining 5 cats lesions completely covered the dorsal cap of the MAO. OKN in the IO-lesioned cats were plotted in the optokinogram. A normal range of TNB in 9 cats fell in

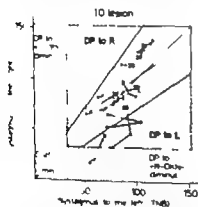


Fig. 2 Optokinogram using TNB as measure in the IO-lesioned cats. The first open circle in the hexagonal area indicates TNB before IO lesion. The subsequent changes of OKN according to time course are indicated by arrows. Each arrow is interrupted with the cat numbers. About week intervals between the one end of an arrow and the other end for an open circle or square and filled circle. The OKN in all the IO-lesioned cat are unaffected and every open square falls within the normal range except for cat no. 14.



Table 1 Normal limits of each parameter of OKN and normal limits of directional preponderance of OKN in 35 normal cats

|                                     | Parameter OKN |              |            |
|-------------------------------------|---------------|--------------|------------|
|                                     | TNB           | OAL          | MES        |
| Mean values                         | 93.1          | 40.8 (°/sec) | 508.1 (μV) |
| Standard deviation                  | 29.7          | 13.5         | 212.4      |
| Normal limits<br>( $m+2SD$ )        | (33-153)      | (13-68)      | (83-933)   |
| Mean values of DP%                  | 6.5 (%)       | 5.1 (%)      | 9.4 (%)    |
| Standard deviation                  | 5.1           | 3.0          | 8.6        |
| Normal limits of<br>DP% ( $m+2SD$ ) | 17            | 11           | 27         |

either IO or SC follow-up studies started from the fourth or the fifth postoperative day on ward at about one week's interval. Alertness was maintained by an intramuscular injection of amphetamine (0.5 mg/kg) about 30 min prior to the electronystagmographic (ENG) test.

OKN was elicited by rotating strips of light projected onto a white screen. The cat was placed within about 65 cm distance from an optokinetic (OK) drum (a cylinder 2 m in diameter and 90 cm high) and its subtended visual angle was surrounded with OK screen. White strips 5 cm wide were rotated at an angular acceleration of  $10^\circ/\text{sec}^2$  for 60 sec. The cats were exposed to two different stimuli applied in both a clockwise and a counter-clockwise direction. As indications for abnormal optokinetic responses we chose the total number of beats (TNB), the OK adaptation limit (OAL) and the maximum eye speed (MES) which was calculated from the calibration curves of the apparatus (Honrubia et al 1971). The mean values and normal limits of each parameter in 35 cats before inflicting lesions are shown in Table 1. An abnormal optokinetic directional preponderance (OKN DP) was established in accordance with the formula (Jongkees & Philipszoon 1964). With L and R representing the left-beating and the right beating OKN responses respectively the percentage OKN DP is

$$\text{DP\%} = \frac{L-R}{L+R} \times 100$$

The normal limits obtained from 35 normal cats are presented in Table 1. Interindividual variation was large. Of three parameters TNB was adopted because there was a linear relationship between the square root of the OKN frequency and the logarithm of the velocity of the OK stimulus (Honrubia et al 1971). The normal limits for DP in TNB obtained from 35 cats were set at 17% (Table 1) and all individual values for TNB were plotted in the optokinogram (Fig. 1) (Mizukoshi et al 1977). This chart represents the normal limits and at the same time gives a survey of conceivable

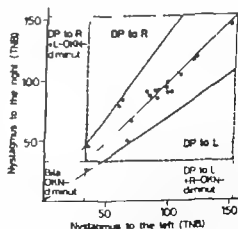


Fig. 1 Optokinogram using TNB as a parameter in 35 normal cats. Clockwise OK stimulus produces OKN to the left, and counter-clockwise OK stimulus OKN to the right. The normal range of TNB is 33-153 nystagmus beats (hexagonal area). The shaded areas indicate OKN DP to the right alone and that to the left alone. The other three areas include bilateral OKN-diminution (lower left square) and unidirectional diminution of OK responses combined with OKN DP.

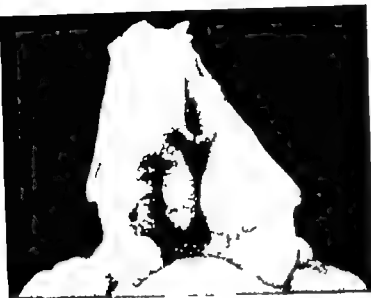


Fig. 3. SC lesions. Group II shows large lesions partially invading the pretectum.

OKN also normal. In cat 15, VS of caloric nystagmus with the quick-phase to the right was reduced by about 10% in light though normal VS was observed in the left-directed nystagmus. This percentage reduction in VS is rather small compared with that for the standard criteria (Kato et al. 1979a). This phenomenon was transient, however. At the 9th postoperative test, VS rate had recovered to the normal range. OKN at this time showed DP to the left (Fig. 3). Histological examination revealed a very small restricted lesion in the SC. Seven of 9 cats were found to belong to Group II, depending upon the extent of the lesion. In these cats, loss of VS was persistently recognized. After inflicting the lesions, the slow-phase velocity of nystagmus with the quick-phase toward the side of the lesions was reduced in light. VS of caloric nystagmus with the quick-phase toward the normal side was lost in light. OKN revealed DP to the left with diminished OK responses to the right as presented in Fig. 3.

In cats 29 and 38, VS of caloric nystagmus reappeared again at the 12th and 1st postoperative test respectively after making the SC lesion. It was then that the IO lesions were inflicted in these cats. VS of caloric

nystagmus however was clearly revealed during the subsequent experimental course. OKN also remained unchanged. OKN of a representative in this series showing loss of VS of caloric nystagmus is presented in Fig. 4. The upper trace in A-F is an ordinary curve (time constant 1.5 sec) and the second trace in each series a derived curve (time constant 0.03 sec). The bottom trace in E and F is a drum speed of OK stimuli recorded with a photocell. Upward and downward arrows in each series mark the moment of onset and end of OK stimulus respectively. A, C and E represent OKN to the left with OK strips rotated to the right. B, D and F show OKN to the right. OKN before the lesions is shown in A and B. OKN after the lesions in C, D, E, and F. At the 9th postoperative test, the left directed OKN showed a slight decrease in response (C) while OKN to the right diminished by 56% (D). At the 26th test after the SC lesion, the right-directed OKN is still reduced by 26% (F) though the OKN toward the left side was nearly normal (E). In this case, OKN showed DP to the left with diminished OK responses to the right after the lesions, and tended to recover to the normal range with the lapse of time (Fig. 3). In this

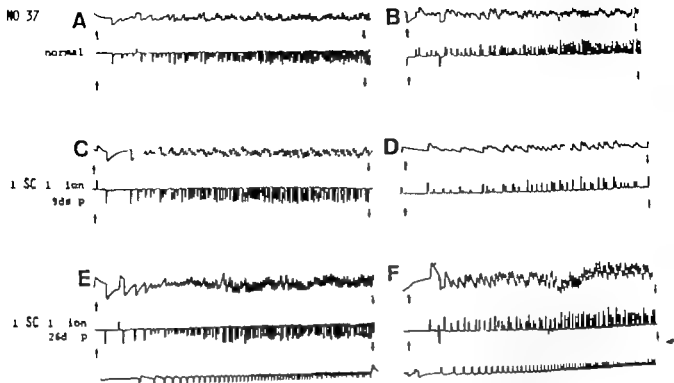


Fig. 4 Effects of left SC lesions upon OKN in a cat representative of Group II. A, B, normal OKN before SC lesions. At the 9th test, left-directed OKN shows a slight decrease in response (C) and OKN to the right is reduced by 56% (D). At the 26th test after lesioning,

right-directed OKN is still reduced by about 76% (F) though OKN toward the left side is near normal (E). Optokinogram in the cat 37 shows OKN-DP to the left with diminished OK responses to the right (Fig. 3).

the hexagonal area (Fig. 2). The first open circle in the hexagonal area indicates TNB before inflicting lesions. The next open square represents TNB recorded about a week later after the IO had been lesioned. The subsequent changes of OKN according to its time course are indicated with arrows, though these are interrupted with the cat numbers. The filled circles represent SC lesions which were inflicted in the cat 35 and 39. About a week intervened between the one end of an arrow and the other end for an open circle or square and a filled circle. Symbols in Fig. 3 signify the same meanings as above. The OKN in all the IO-lesioned cats were unaffected and every open square fell in the normal range except for cat no. 35. In cats 35 and 39 the SC was lesioned subsequently on the 12th and 14th day from the IO lesions. In these two cats, VS of caloric nystagmus with the quick phase to the right was lost after the SC had been lesioned. OKN revealed DP to the left

with diminished OK responses to the right in the 35 and DP to the left in cat 39 (Fig. 7).

### SC lesion

Cats with SC lesions were classified into the following two groups depending upon the extent of the lesions. Group I with lesions in the very small restricted area and Group II with lesions encroaching in almost all the anterior half of the SC and extending rostrally to a part of the pretectum. In all the SC-lesioned cats the eye movements were normal and the pupils showed normal response to light. When TNB was used as a parameter, the optokinogram as illustrated in Fig. 3 was obtained in all the SC-lesioned cats. The overall tendency of OKN after making the lesion in the SC showed DP to the left with diminished OK responses to the right except for 2 cats (nos. 14, 15) belonging to Group I. In cat no. 14, VS of caloric nystagmus was clearly observed throughout the whole experimental course and

urde an III Katzen OKN nach Läsion der unteren Olive und des Colliculus superior wodurch der Flocculus das visuelle Signal empfängt, untersucht. Nach Läsion der unteren Olive wurde die visuell ausgelöste Suppression des kalorischen Ny. begrenzt, konnte noch beobachtet und OKN abgelesen von einer Katze, auch normal ausge-  
 124 Nach Colliculusläsion wurde der Effekt des Lichts auf den kalorischen Nystagmus an 7 von 9 Katzen nicht beobachtet und OKN in diesen Katzen zeigte das Richtungsüberwiegen nach links und die Verminderung oder den Ausfall nach rechts. Von diesem Resultat darf man daher ableiten, daß Colliculus superior als präfixoculärer Kern eine wichtige Rolle spielt.

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cat loss of VS lasted for 75 days Fig 5 showed lesions in the SC Rostrally placed lesions in the SC invaded the pretectum partially

## DISCUSSION

The flocculus exercises gain control of VOR by using visual information which has the effect of improving the stabilization of retinal images during head movement (Ito et al 1974) When the fixed slit light is presented to an eye with the rabbit mounted on the turntable the horizontal movement of that eye during head rotation is enhanced in comparison with that in darkness In contrast, the eye movement is reduced by a slit light moving in phase with the turntable This gain control is effectively abolished when the flocculus is extirpated (Ito et al 1974)

In the rhesus monkey caloric nystagmus is suppressed in light as compared with that in total darkness (Takemori & Cohen 1974a) This visually induced suppression of caloric nystagmus also occurs in the direction of stabilizing retinal images of the visual surroundings In fact visual suppression of caloric nystagmus is lost after the flocculus is lesioned (Takemori & Cohen 1974b) Furthermore it is noticeable that OKN is also disturbed in monkeys with lesions in the flocculus (Takemori & Cohen 1974b) Two possible explanations come to mind for the release of visual suppression of caloric nystagmus the one is a disturbance of the gain control system of VOR exerted directly by the flocculus and the other is the disturbance of the primary oculomotor system such as gaze holding smooth pursuit eye movements and optokinetic responses The latter is suggested by the present experiment showing that in cats with lesioned SC loss of VS is recognized and OKN disturbance is evident

The dorsal cap of the MAO could be one of the main relay nuclei to mediate visual signals to the flocculus (Alley et al 1975 Takeda & Mackawa 1976) In our previous experiment

electrolytic lesions were placed at various antero-posterior levels of the IO (Kato et al 1979b) In particular the dorsal cap of the MAO was confirmed by responses to both light-guided and antidromic field potentials with a bipolar stimulating electrode inserted into the contralateral flocculus Electrolytic lesions of the dorsal cap resulted in complete loss of a late component of the field potentials from the flocculus Even in the cats prepared in this fashion VS of caloric nystagmus was clearly demonstrated (Kato et al 1979b) Furthermore in the present experiment, the IO lesions had no effect upon OKN These findings are strongly supported by many reports suggesting that the IO is not related to the temporary modification of the VOR by visual stimuli (Ito 1976 Kawasaki et al 1978 Kato et al 1979b) An electrical stimulation of the SC evoked pronounced mossy fiber responses in the flocculus even after severance of the accessory optic tract Furthermore the early component of field potentials (Machawa & Takeda 1975) was reduced after SC lesions (Kawasaki et al 1978) On the basis of the present findings showing that the loss of VS and disturbance of OKN are clearly demonstrable in SC lesioned cats it may be assumed that the SC is an important relay nucleus to the flocculus for conveying the visual signals responsible for VOR gain

## ACKNOWLEDGEMENTS

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## ZUSAMMENFASSUNG

Der Verlust der visuell ausgelösten Suppression des kalorischen Nystagmus wurde nach Läsion des Flocculus in den Affen demonstriert, dann wurde optokinetischer Nystagmus (OKN) auch gestört In diesem Experiment

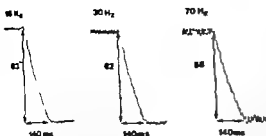


Fig. 1. Changing the low-pass filters from 15 to 30 and to 70 Hz does not significantly influence the velocity of the saccade as calculated from the recordings.

15 Hz (Mingograph M 81, Siemens-Elema, Stockholm, Sweden) served as a recorder.

Before the selection of the filter was made the rise time of the amplifier was tested by using square waves and ramp signals corresponding to different angular velocities of the eyes. When filters of 15, 30, 70 or 700 Hz were used, the amplitude, the shape and the duration of these "electrical saccades" did not change noticeably when signals with a rise time of 50 ms or longer were applied. To exclude high frequency noise we could therefore use the 15 Hz filter without any distortions of the signal (see also Fig. 1).

The paper was fed at 100 mm/s to

secure the accuracy in the analysis of recordings for events occurring within 10 ms (1 mm). In the recording an angular deviation of the eyes of 1° corresponded to 1 mm.

The patient was sitting comfortably in a chair. The head was mechanically supported and the position of the head adjusted (eye-ear axis horizontal) for each subject before any measurements were undertaken. Light diodes were placed 120 cm in front of the subject at angles of 5°, 10°, 20° and 30° to the right as well as to the left of the mid-line. Calibration was made with the subject looking alternately at the diodes which were 20° and 60° apart.

**Saccades.** The tests were performed in darkness. The targets consisting of light diodes of different colours were therefore easy to distinguish. At least 10 saccades at each of the amplitudes 5°, 10°, 20°, 30°, 40° and 60° were performed. The light diodes were then switched off and the subject was asked to look first with his eyes open and then to try to look also with his eyes closed at the diodes 10° apart, 20° apart, and finally at those 60° apart.

**Caloric test.** A routine caloric test (Hen-

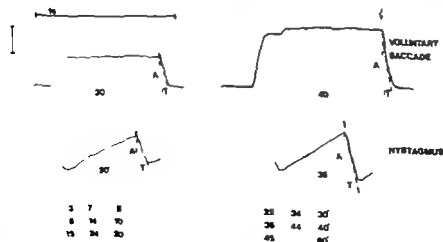


Fig. 2. The peak velocities of rapid eye movements are measured and classified according to amplitude of the eye movement. The tangent at peak velocity is visually

determined for the recording and is calculated ( $p = A/T$ ) by using the amplitude ( $A$ ) and duration ( $T$ ) at peak velocity ( $p = 17$ ).

## VELOCITY PATTERNS OF RAPID EYE MOVEMENTS

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**Abstract** The peak velocities of saccades and fast phases of nystagmus were examined and compared in 20 healthy subjects. The peak velocities of both types of eye movements increased with increase of amplitudes. The saccades were found to be fastest in light slower in darkness and slowest behind closed eyelids. The peak velocities of the quick phases of optokinetic and of vestibular nystagmus were found to be the same. Fast phases of optovestibular (optic as well as vestibular stimulation) nystagmus produced significantly higher peak velocities than the two others. At the same amplitude and during the same visual conditions the saccades were significantly faster than any type of fast components of nystagmus. The difference in velocity between voluntary and reflexive eye movements is possibly related to differences in antagonistic activity during these eye movements, but also to specific synaptic events during the voluntary action.

1969 Ron et al 1972) who neither in man nor in intact alert monkey found differences between saccadic velocity and velocity of fast phases of nystagmus.

As these findings were in contrast to our observations the present study of the velocity of the two types of rapid eye movements was undertaken.

The aim of this paper will be to determine the velocities of the fast phases of nystagmus of 20 normal subjects exposed to rotation to caloric stimulation as well as to optokinetic stimuli for comparison with the velocity of the voluntary saccades at corresponding amplitudes.

There are two main groups of rapid eye movements: the saccades and the fast phases of nystagmus. Whereas the saccades can be triggered voluntarily or spontaneously by peripheral visual stimuli the fast phases of vestibular and of optokinetic nystagmus are triggered reflexively secondary to the slow phase of nystagmus.

All rapid eye movements seem to utilize the same pontine neuronal network located in the parabrachial reticular formation (Cohen & Feldman 1968; Cohen & Komatsuzaki 1972; Cohen et al 1973; Henn & Cohen 1975) where the position of the eyes is adjusted to the position of the target (Robinson 1975). A similar firing pattern is thus found in these neurons during saccades as well as during fast phases of nystagmus (Keller 1974; Henn & Cohen 1975).

This would support findings by several authors (Dichgans et al 1969; Nauck et al

## MATERIAL AND METHODS

The material consisted of 20 volunteers with normal hearing and vision. They had no history of neurological disease and a routine otoneurological examination revealed free eye movements. Ten were males and 10 females and their mean age was 48 years with a range of 23 to 72 years. An electro-oculographic (EOG) technique with commercial electrodes and electrode jelly (Medicotest<sup>®</sup>) was used.

In the horizontal plane the mean of the movements of the right and left eye was recorded by the use of conventional electrodes horizontally disposed while the recording of vertical eye movements was made for each eye separately. A DC-coupled ink writer with an upper cut-off frequency of

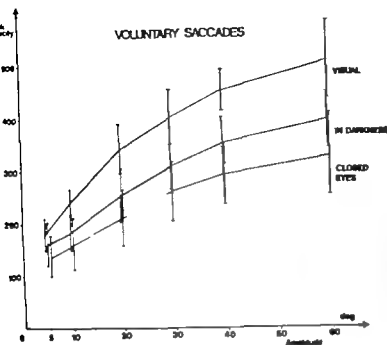


Fig. 3. Mean peak velocities with standard deviations of voluntary saccades when performed in visual conditions, in darkness and in darkness behind closed eyelids at different amplitudes.

At 20° amplitudes the mean peak velocity of the saccades in light was about 340°/s, in darkness about 250°/s and behind closed eyes about 210°/s (Fig. 3). From these values the peak velocities increased with increasing amplitudes of the saccades and at amplitudes of 60° were 500°/s, 400°/s and 320°/s respectively (Fig. 3).

Saccades were characterized by highly variable velocities when performed without visual control. Sometimes efforts to produce saccadic eye movements with closed eyes resulted in recordings appearing more as slow deviations of the eyes than as fast saccadic eye movements (Fig. 4). In darkness, over shooting saccades were very common especially at the amplitudes of 10° and 20°.

There was further a considerable inter and individual variation in saccadic velocity. Since this study was focussed on the mean values of a whole group of subjects examined in standardized visual conditions no further interest was paid to either inter or intrasubject variations.

We found no difference in the peak ve-

locities of saccades between different age groups (Fig. 5). Young subjects had no higher saccadic velocity than older ones. In fact a subject aged 72 had faster saccades than another subject aged 22.

The rapid return eye movements following the pursuit eye movements were analyzed in five subjects. These rapid eye movements had the same amplitude-velocity relationship as visual saccades. Hence we considered these movements identical to the saccadic ones.

*Fast phases of nystagmus.* We found no difference in the peak velocities of the fast phases of caloric nystagmus, whether induced by irrigation of the right or of the left ear by cold or by warm water. These peak velocities were as were the velocities of the saccades strongly dependent upon the amplitude of nystagmus as shown in Fig. 6. Moreover the fast phases of nystagmus were equal whether caused by rotation or by caloric stimulation. The peak velocities of the fast phases of nystagmus induced by rotation at amplitudes of 20° were about



Table I Mean peak velocities with spreads of voluntary saccades at amplitudes of 20° and of 60° in two groups of subjects tested in two laboratories

|         | No | Age (years)<br>mean and range | Laboratory | Peak velocity of saccades at |                                  |
|---------|----|-------------------------------|------------|------------------------------|----------------------------------|
|         |    |                               |            | 20°<br>mean $\pm$ S D        | 60°<br>mean $\pm$ S D            |
| Group A | 20 | 48.4 (23-72)                  | I          | 336° s $\pm$ 42° s           | 495° s $\pm$ 85° s <sup>-1</sup> |
|         |    |                               | II         | 327° s $\pm$ 61° s           | 480° s $\pm$ 96° s               |
| Group B | 12 | 45.0 (18-70)                  | II         | 316° s $\pm$ 67° s           | 465° s $\pm$ 96° s               |

riksson et al 1972) was conducted in darkness with the subject's eyes open.

**Rotation test** The rotation test was performed in complete darkness by acceleration of the subject within one second to a constant velocity of 120° s<sup>-1</sup>. After one minute of rotation the chair was brought to a stand still within one second. The perrotatory as well as the postrotatory nystagmus was recorded with the eyes open.

**Optokinetic test** The optokinetic test was performed with the subject inside a striped drum rotating around the patient. The drum was accelerated for 10 seconds to a constant velocity of 90° s<sup>-1</sup> and the calculations were made at velocities of the drum at 90° s<sup>-1</sup> as well as somewhat below this value.

**Optovestibular test (optokinetic + rotation stimulus)** In this test the subject was rotated inside the optokinetic drum with his eyes open. Using this technique eye movements were recorded resulting from a combination of vestibular and optokinetic stimuli. The physical properties of the acceleration in this combined test were the same as those in the rotation test in darkness.

**Evaluation of recordings** The peak velocities were measured by estimating the tangent for rapid eye movements; this usually occurred at the beginning of the eye movement. The saccades and the fast phases of nystagmus were classified into different groups according to their amplitudes (Fig. 2). The means and standard deviations of the velocities of the eyes in the saccades and in

the fast phases of the various types of nystagmus were determined for each individual and for each amplitude (10° 20° 30° 40° and 60°).

To assess the stability of the mean saccadic velocity within the same group of normals this velocity was determined on two different occasions with an interval of about one hour. Two different sets of recording devices in two different laboratories were used.

To study the variation between two different groups of normals another group of 17 subjects was examined with a similar age distribution as the subjects in the main study (Table I).

## RESULTS

**Saccades** The mean peak velocities of the saccades at amplitudes of 20° and 60° for the same group of subjects in two different laboratories are provided in Table I. Despite the fact that the recordings were made in different laboratories with an interval of one h the mean peak velocities were very close. Also when two separate groups of subjects were examined the mean peak velocities were quite similar (Table I).

The amplitude—peak velocity relationship of saccades light in darkness and with closed eyes is shown in Fig. 3. Saccade peak velocity was dependent not only upon the amplitude but also on the visual condition. With a given visual condition an increase in amplitude produced an increase in the peak velocity; the function being curvilinear.

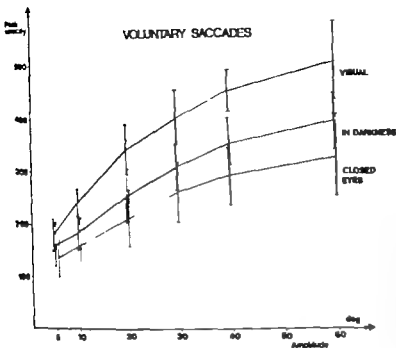


Fig. 3. Mean peak velocities with standard deviations of voluntary saccades when performed in visual conditions, in darkness and in darkness behind closed eyelids at different amplitudes.

At  $70^\circ$  amplitudes the mean peak velocity of the saccades in light was about  $340^\circ/\text{s}$  in darkness about  $250^\circ/\text{s}$  and behind closed eyes about  $210^\circ/\text{s}$  (Fig. 3). From these values the peak velocities increased with increasing amplitudes of the saccades and at amplitudes of  $60^\circ$  were  $500^\circ/\text{s}$ ,  $400^\circ/\text{s}$  and  $370^\circ/\text{s}$  respectively (Fig. 3).

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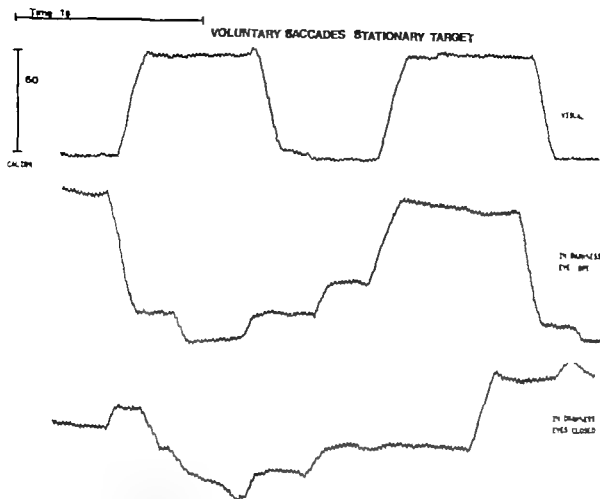


Fig. 4. A typical recording showing voluntary saccades when performed in visual conditions in darkness eyes open and in darkness behind closed eyelids.

$215^{\circ} \text{ s}^{-1}$ . Velocities at higher amplitudes could not be determined as such nystagmus is rare in adults (Tibbling 1969).

The amplitudes of OKN (optokinetic nystagmus) were generally smaller usually below  $20^{\circ}$ . By increasing the velocity of the stimulus the amplitude of nystagmus tended to decrease. Hence for optokinetic nystagmus we obtained only a few measuring points for higher amplitudes than  $20^{\circ}$ . The amplitude-velocity relationship for the fast phases of OKN is also presented in Fig. 6. For the measurable amplitudes the OKN fast phase velocities closely followed the corresponding curves for the fast phase of vestibular nystagmus.

By rotating the subjects within the optokinetic drum with the subject's eyes open

and in light (combining visual and vestibular stimuli) the peak velocity of the fast phase of nystagmus increased (Fig. 6). The peak velocity of the fast phases of the combined nystagmus about  $260^{\circ} \text{ s}^{-1}$  and the amplitude of  $20^{\circ}$  against  $215^{\circ} \text{ s}^{-1}$  at the same amplitude for pure vestibular or pure optokinetic nystagmus.

*Comparison of velocities of saccades with fast phases of nystagmus.* Fig. 7 shows a summarising diagram of the peak velocities of saccades and of fast phases of nystagmus with eyes open in light or in darkness. With the same visual condition and at the same amplitude the saccades were always faster than the fast phases of any type of nystagmus here examined. The difference was more pronounced at greater amplitudes.

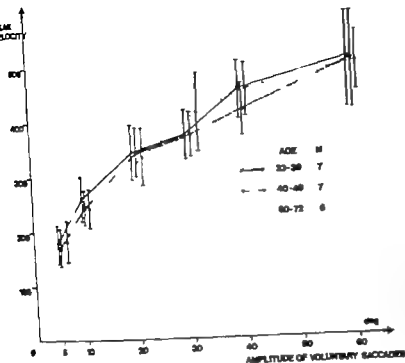


Fig. 5. Mean peak velocities with standard deviations of visual voluntary saccades in three different age groups at different amplitudes.

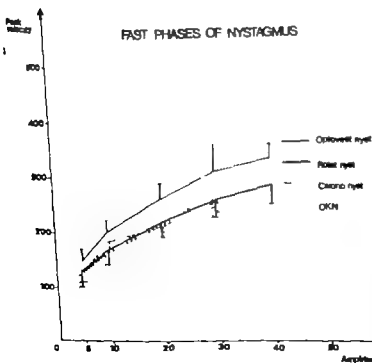


Fig. 6. Mean peak velocities and standard deviations of fast phases of optokinetic nystagmus (—), of rotatory nystagmus (—), of caloric nystagmus (—) and of optokinetic nystagmus (—). The spreads are presented only in direction

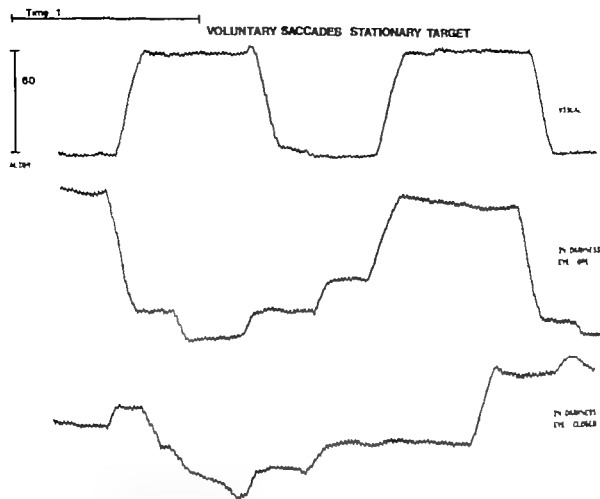


Fig. 4 A typical recording showing voluntary saccades when performed in visual conditions, in darkness eyes open and in darkness behind closed eyelids

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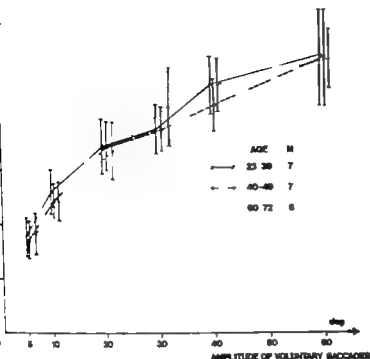


Fig 5 Mean peak velocities with standard deviations of visual voluntary saccades in three different age groups at different amplitudes.

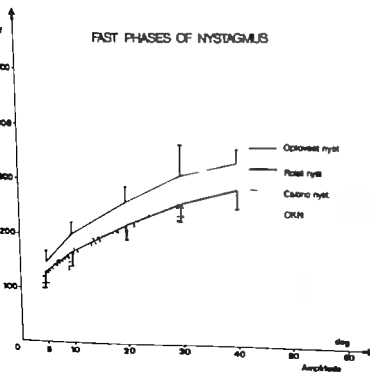


Fig 6 Mean peak velocities and standard deviations of fast phases of optovestibular nystagmus (—) of rotatory nystagmus (---), of caloric nystagmus (···) and of optokinetic nystagmus (- · -). The spreads are presented only in direction

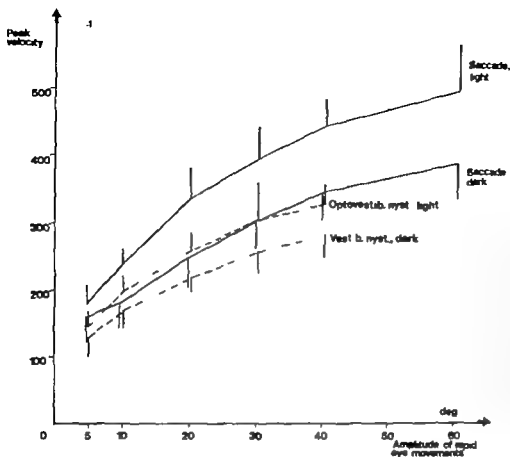


Fig 7 A summarizing diagram showing the difference between velocities of saccades in light and of fast phases of optovestibular nystagmus. The difference is also shown between the velocities of the saccades in dark and the velocities of fast phases of rotatory nystagmus (vest. nyst. dark). The parameters are as in Figs. 3 and 4

## DISCUSSION

It must be stressed that during the saccade the velocity is not constant. The peak velocity is usually reached somewhat before the middle of the saccades after which the velocity gradually declined (Westheimer 1964, Collins 1975). Hence the saccades represent typical ballistic movements of the eyes (Robinson 1964). Our reason for measuring peak velocity instead of duration or mean velocity of saccades was due to an observation by Keller (1974) who found a highly synchronous firing pattern within the pontine neurons during peak velocities. We have therefore assumed that peak velocity should be more sensitive to disturbances in clinical connection than other parameters of the saccades (Baloh et al 1975).

The difference in velocity between saccades and fast phases of nystagmus has not been reported earlier. The complexity in oculomotor mechanism makes the observation difficult to explain. Thus the cortical

mechanism involved in visual saccades is not yet fully understood. Single unit recordings have shown activity during saccades in the neurons of Brodmann's area 8 (Buzzi 1968, Buzzi & Schiller 1970), area 7 (Straschill & Schick 1974, Mountcastle et al 1975, Lynch et al 1977) but also in areas 17, 18, 19 (Hubel & Wiesel 1965, Orban & Callens 1977a, b). The importance of these centres is further supported by lesion studies (Pasik & Pasik 1964, Latt & Cowey 1971, 1972).

The efferent pathways from these three cortical fields descend to neurons in the pontine reticular formation (Astruc 1971). Through other pathways the same pontine neuronal network is also activated during the fast phases of vestibular and optokinetic nystagmus. From there oculomotor impulses are channeled to the oculomotor nuclei through final pathways common to all different types of rapid eye movements (Henn & Cohen 1975). This conception of a final pathway common to the oculomotor system has sup-

ported the idea that all types of rapid eye movements should be equally rapid. Our observations to the contrary may have some support in previous literature also.

Thus the normal contraction of the antagonistic muscles present only during the fast phase of nystagmus (Shimazu 1972) might explain a difference in velocity between fast phases and saccades. Further the "slow" saccadic velocities noticed e.g. in Huntington's chorea, has been believed to be due to lack of proper inhibition of antagonistic muscles (Starr 1967).

One other factor which seems to be of importance is that saccades are initiated voluntarily but fast phases of nystagmus are deiced automatically and secondarily to low phases. During voluntary saccades the subject is alert and active performing a task. While during fast phases of nystagmus the subject is passive. The higher velocity of the saccades might therefore at least in part be explained by a more powerful excitation of pontine neurons caused by volition or related alertness caused by this volition.

## ZUSAMMENFASSUNG

Willkürliche schnelle Augenbewegungen (Saccaden) und reflexbedingte schnelle Augenbewegungen (schnelle Nystagmusphasen) werden bei 20 gesunden Personen untersucht und miteinander verglichen. Die Geschwindigkeiten beider Augenbewegungstypen steigen mit einer Zunahme der Amplituden an. Es wurde gefunden, daß die willkürlichen Augenbewegungen, die Saccaden, ein Licht am schnellsten, langsamer in Dunkelheit und am langsamsten in einer geschlossenen Augenlidern sind. Während die Geschwindigkeiten der schnellen Phasen von optokinetischen und vestibulären Nystagmen identisch sind, erreicht die schnelle Phase von opto-vestibulären Nystagmen (sowohl optische als auch vestibuläre Stimulation) signifikant höhere Geschwindigkeiten als beide anderen Typen. Bei gleicher Amplitude und unter gleichen visuellen Bedingungen waren die Geschwindigkeiten der willkürlichen schnellen Augenbewegungen (die Saccaden) signifikant höher als die schnellen Komponenten reflexbedingter Augenbewegungen.

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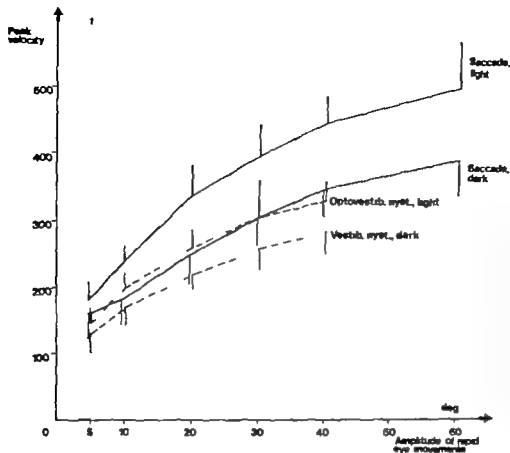


Fig 7 A summarizing diagram showing the difference between velocity saccades in light and dark and the fast phases of optokinetic nystagmus. The difference is also shown between velocities of the saccade in dark and the velocity of the fast phases of vestibular nystagmus (vest. nyst. dark). The parameters are as in Figs. 3 and 4

## DISCUSSION

It must be stressed that during the saccade the velocity is not constant. The peak velocity is usually reached somewhat before the middle of the saccades after which the velocity gradually declined (Westheimer 1954 Collins 1975). Hence the saccades represent typical ballistic movements of the eyes (Robinson 1964). Our reason for measuring peak velocity instead of duration or mean velocity of saccades was due to an observation by Keller (1974) who found a highly synchronous firing pattern within the pontine neurons during peak velocities. We have therefore assumed that peak velocity should be more sensitive to disturbances in clinical connection than other parameters of the saccades (Baloh et al 1975).

The difference in velocity between saccades and fast phases of nystagmus has not been reported earlier. The complexity in oculomotor mechanism makes the observation difficult to explain. Thus the cortical

mechanism involved in visual saccades is not yet fully understood. Single unit recordings have shown activity during saccades in neurons of Brodmann's area 8 (Bizzi & Schiller 1970), area 7 (Straschewsky & Schick 1974, Mountcastle et al 1977), but also in areas 17 and 19 (Hubel & Wiesel 1965, Orban & Callot 1977a, b). The importance of these areas is further supported by lesion studies (Pascual-Leone & Pasik 1964, Latt & Cowey 1971, 1977).

The efferent pathways from these cortical fields descend to neurons in the pontine reticular formation (Astruc 1977). Through other pathways the same pontine neuronal network is also activated during the fast phases of vestibular and optokinetic nystagmus. From there oculomotor impulses are channeled to the oculomotor nuclei through final pathways common to all different types of rapid eye movements (Henn & Cohen 1975). This conception of a final pathway common to the oculomotor system has

## THE MECHANISM OF PHYSIOLOGICAL HEIGHT VERTIGO

### I Theoretical Approach and Psychophysics

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**Abstract.** A theory is presented supporting geometrical explanation of physiological height vertigo as a distance vertigo created by visual destabilization of posture when the distance between the observer and visible stationary contrasts becomes critically large. Though height vertigo is generally regarded as a psychopathological process, we hypothesize that it might instead result from an sensory mismatch when visual information is at variance with vestibular and proprioceptive inputs. Psychophysical experiments confirming the hypothesis revealed that: 1) height vertigo is clearly related to body position, being the greatest in the upright stance; 2) it is the eye-object distance rather than the direction of gaze which is critical; 3) there is saturation of height vertigo magnitude. Subjective vertigo increases with increasing altitude only below 20 metres. Physiological distance vertigo must be distinguished from psychological acrophobia. Its potential consequences may be ameliorated by strategies derived from knowledge of its mechanism such as providing nearby stationary contrasts in the peripheral visual field.

Height vertigo, a visually induced syndrome commonly experienced on top of high structures is manifested by a subjective instability of posture and locomotion coupled with a fear of falling and vegetative symptoms. It is similar to motion sickness in that both are acute physiological symptoms which spontaneously remit after termination of the inducing stimulus situation. Also in analogy to motion sickness, habituation to height vertigo may occur through repeated exposure such as observed in steeplejacks, roof workers and tight rope artists, who achieve a remarkable degree of postural balance with seeming insensitivity to height.

Height vertigo generally has been attributed to psychopathological processes such as neurotic acrophobia or neurasthenic dizziness. We believe that height vertigo does not simply represent an unpleasant visual epiphenomenon but rather is a meaningful warning signal to the body for withdrawal from stimulus situations which cannot be adequately perceived in terms of space constancy necessary for postural control. We are postulating a physiological mechanism for height vertigo, separate and distinct from purely cognitive or psychological factors. Psychophysical data are presented in this paper and posturographic data in a subsequent study (paper II, Bles et al 1979) which support a simple geometrical explanation for physiological height vertigo as being a distance vertigo.

We reasoned that a visual destabilization of postural balance would induce height vertigo when the distance between the observer and visible stationary contrasts became critically large. A brief review of the visual contribution to balance in man will be presented as a necessary prelude to assist in understanding our hypothesis and experimental design.

Optimal balance requires the continuous evaluation of the reafferent sensory consequences of self-generated body movements. There are three main loops which control posture: 1) vestibular, 2) somatosensory (proprioceptive) and 3) visual. Each control loop has its own frequency range of optimal func-

- tions for operations within extrapersonal space. *J Neurophysiol* 38: 871
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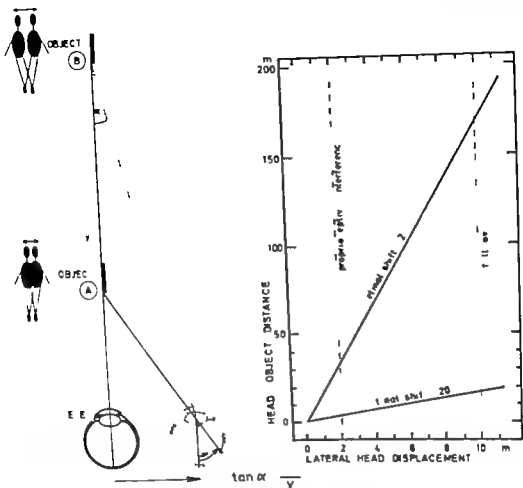


Fig. 1. Geometrical analysis indicates that in order to be visually detected, body sway must increase with increasing distance between the eyes and the nearest stationary constraints. Angular displacements  $\alpha$  on the retina, caused by lateral head displacement, are smaller the greater the distance to the object. Diagram shows rela-

tionship between head-object distance  $y$  and lateral head displacement, for given retinal displacement thresholds of either 2 or 20 minutes of arc. However, because postural regulation involves multiloop control, additional proprioceptive cues may alter sway amplitudes as well.

1955) a normal lateral head sway of 2 cm would be subthreshold at a distance of about 3 m. This should lead to a perceptual conflict as the vestibular and somatosensory receptors sense a body shift which the visual system cannot detect. The conflict might be resolved by increasing the postural sway and thereby reactivating visual control. For a simple loop control a relationship between distance and sway amplitude could be expected ( $\tan \alpha = y$ ) with the gain dependent on the retinal movement detection threshold. However

with the lower limbs close together at free stance one falls over at a head sway more than 10 cm. Thus at an eye-object distance of 15–20 m a maximal body sway would produce retinal angular deviations ( $\alpha$ ) less than 20 mm of arc according to the trigonometrical model. Since we are dealing with a multiloop control of postural balance it can be assumed that with increasing sway amplitudes the particular sensory weight of the somatosensory and vestibular afferences becomes greater increasing their contribution to pos-

tion. With some overlap among the ranges they cover the entire range encountered in daily life.

Vision plays a major role in postural stabilization. It attenuates self-generated sway by 50–100% (Edwards 1946, Travis 1945) and its effect on postural stability accounts for the significance of the Romberg test in clinical examinations (Romberg 1846). The role of vision is particularly apparent in patients with diseases involving the proprioceptive joint and muscle afferents, as in *tabes dorsalis* (Duchenne 1958, Frenkel 1907) or in patients suffering from postconcussional dizziness (De Wit & Bles 1975) and is also important to a lesser degree in patients with cerebellar (Dichgans et al 1976) or vestibular lesions (Barré 1949, De Haan 1959, De Wit 1973). Under experimental conditions vision may have a strong destabilizing effect on posture, particularly when visually perceived motion does not adequately correspond with the actual body shift sensed by the vestibular and somatosensory systems. Observations of an optokinetically induced body sway have been reported in the older literature by Fischer & Kornmüller (1930). Extensive experiments on that phenomenon have been performed with movements of an artificial surround as circular motion about the vertical Z axis (Kapteyn & Bles 1977), roll motion about the line of sight (Dichgans et al 1976), a sinusoidally tilting room (Bles et al 1977) and linear motion of the seen environment (Lee & Aronson 1974, Berthoz et al 1975). In these instances there was an optokinetically induced perception of apparent self-motion opposite in direction to the pattern motion. The postural reflexes compensate for this subjective body shift resulting in a measurable body tilt in the direction of the motion pattern. These optokinetically induced postural reactions require stimulation of the entire visual field or substantial portions of the periphery. This corresponds to the stimulus characteristics of visually induced vections. Dynamic spatial orientation and postural control rely mainly on information from

the retinal periphery (Brandt et al 1973, Dichgans & Brandt 1978).

Studies on children revealed that the visual contribution to postural stabilization participates rather late after the acquisition of upright stance and gait (Brandt et al 1976). Furthermore, the calibration of the three main loops (vestibular, somatosensory and visual) occurs sequentially, is mutually interactive and remains plastic throughout life. In order to maintain postural stability in the upright position, afferent signals must be generated as an input for compensation of natural fore-aft and lateral body sways. The measurable sway path of the centre of gravity is greater in the fore-aft than in the lateral direction. Fore-aft sway is comparable to the motion of an inverted pendulum as a first approximation (Nashner 1970), whereas lateral sway is horizontal over several centimetres (parallel shift) because of the mechanical joint relationship of the lower limbs, pelvis and vertebral column (Kapteyn 1973). A lateral sway causes a shift of the visible surround on the retina when the eyes are stationary. The importance of retinal image motion in stabilizing posture is supported by experiments utilizing stroboscopic surround illumination. Visual stabilization was practically absent at strobe frequencies up to 6 Hz (Amblard & Cremieux 1976).

### *Hypothesis*

Simple geometrical analysis indicates that body sway must increase with increasing distance between the eyes and the nearest stationary contrasts in order to be visually detected (Fig. 1). Angular displacement  $\alpha$  on the retina is smaller, the greater the distance  $Y$  to the object becomes. Since the natural lateral head sway is about 2 cm in amplitude (Kapteyn 1973), an important determination would be the specific eye-object distance at which this sway amplitude could not be visually detected. Since an angular displacement of the visual scene of 20 min of arc is necessary for detection by the paracentral and peripheral parts of the retina (Aubert 1886, Leibowitz

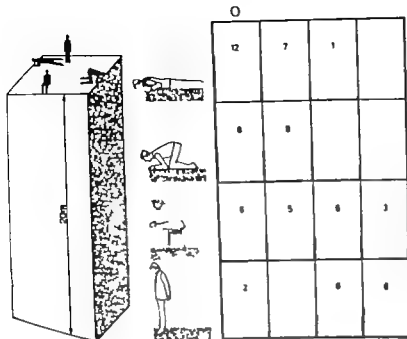


Fig. 3 Magnitude of subjective height vertigo as related to body position, being the greatest with upright stance where maintaining postural balance is comparatively most difficult. Scalings (0 = none = moderate ++ = medium +++ = severe height vertigo) of 20 volunteer subjects not particularly susceptible to height vertigo

2. Height vertigo should be induced either by downward or upward gaze (provided visual conditions are held constant) because it is the distance rather than the visual direction that is critical (psychophysical experiment)

3. Height vertigo should appear at a distance of about 3 m, should increase with increasing distance and saturate at a height below 15–20 m where it should be maximal in intensity (psychophysical and posturographic experiments)

4. Nearby stationary contrasts in the periphery of the visual field should improve the height vertigo sway because the retinal periphery dominates postural balance (posturographic experiments)

5. Extreme head tilt (by which the otoliths are brought out of their optimal working range) or additional disturbances of the somatosensors (Standing on a soft foam rubber platform) should enhance the height vertigo sway because the 'false visual signal' should be accorded a disproportionately greater influence (posturographic experiments)

The psychophysical experiments were per-

formed under natural conditions on high buildings. We were aware of standardization problems which might result by deviating from usual laboratory procedures but felt that field conditions were necessary for proper validation of our hypothesis.

## EXPERIMENTS AND RESULTS

### *Experiment 1 Influence of body position*

Twenty healthy volunteer subjects (10 male 10 female average age 30) who were not particularly susceptible to height vertigo and were unfamiliar with the hypothesis took part in this experiment. They were led separately to the extreme outer edge of the flat roof of a building 20 m high (Fig. 3). In a random order they assumed four different positions, upright stance unsupported sitting, positioning on elbow and knees and lying position. They were required to look down for a period of 1 min and to scale the overall distressing effects or height vertigo experienced in each condition. Using a simple scaling method they had the choice between: none (–) moderate (+) me-

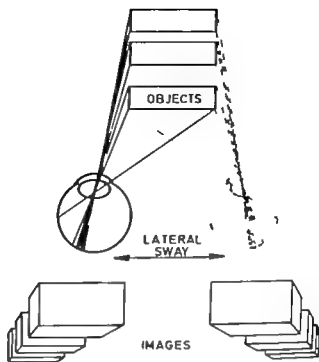
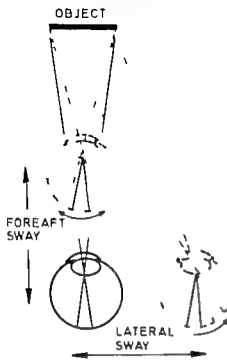


Fig 2 Differential effects of lateral and fore-aft head sway on retinal image shift of a stationary object. Geometrical representation of this shift for two-dimensional

and three-dimensional objects (absolute and relative motion parallax respectively)

tural stabilization. Experiments with the sway ing room demonstrated that optokinetically induced head sway saturates at amplitudes greater than 2 cm which is indicative of proprioceptive interference (Bles et al 1977).

The trigonometrical model developed for afferent motion perception during lateral horizontal body sway must also be valid for afferent motion perception since the thresholds of movement detection are about the same with eye pursuit and stabilized retinal images. It must also hold for fore-aft sway in which the retinal images vary in size with the sway (Fig 2). Involvement of eye torsion—in which the retinal shift is independent of the distance between eye and object—can be consequential for posture in case of great lateral (rotational) sway amplitudes only.

It seems reasonable to conclude that the displacement angle on the retina, dependent as it is upon distance, cannot be a determinant of the net compensatory sway. There must be higher order depth constancy mechanisms similar to those for size and motion constancy involved in compensating for the retinal de-

ficiency. This is evident in a normal structured surround when the body sways in front of several stationary objects at different distances. Here monocular and binocular depth cues as well as motion parallax may be used to stabilize posture (Fig. 2). The gain of the motor reaction to a visually sensed sway requires a precise multisensory calibration based on all mechanisms subserving self motion perception. The retinal shift of about 20 min of arc seems to be a minimal requirement for the visual afferent contribution.

For the validation of our hypothesis that height vertigo is based on visual destabilization of free stance when the distance between eye and object becomes critically large, a number of propositions must be demonstrated with psychophysical (paper I) and posturographic (paper II) experiments.

1. The occurrence of height vertigo should be clearly related to body position. It should be the greatest with the erect stance when maintaining balance is most difficult and minimal in the lying position (psychophysical experiment).

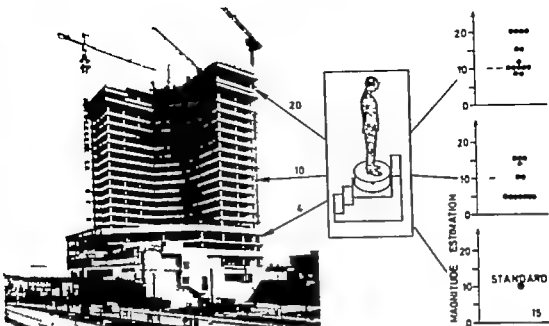


Fig. 5. Saturation of subjective height vertigo at increasing altitudes (20, 10 and 4 metres: 4th, 10th, and 20th floor respectively). Magnitude estimations of 15 subjects

were not significantly different for the three altitudes. The fourth floor (20 metres) served as the standard situation (modulus with an arbitrary value of 10)

desired perceptual cues provided by windows and visible ceilings were prevented by restriction of peripheral vision to a total field of 120 degrees in diameter. Subjects were blindfolded and taken in an outside elevator to the fourth floor at an altitude of approximately 20 m. Thereupon via a staircase they stepped onto the stabilometer platform which stood adjacent to the outer wall of the building. The blindfolds were removed and the subjects suddenly exposed to the height vertigo inducing situation during which they were required to look down for a period of one minute. A security belt was used as a precautionary measure. The fourth floor condition served as the standard situation for further magnitude estimations of subjective vertigo (modulus with an arbitrary value of 10). Posturographic measurements were performed of the fore-aft and lateral body sway with eyes closed and eyes open as well as obtaining the subject's scalings of height vertigo seventy. All's were then taken from the fourth to the twentieth and finally to the tenth floor representing an alti-

tude of about 100 and 50 m respectively. Technical factors within the building site prevented randomization of the order of exposure to the different heights.

As depicted in Fig. 5 statistically there was no significant difference among the height vertigo intensities experienced at altitudes of 20, 50 or 100 m, although some S's gave greater estimations on the twentieth floor. For most subjects vertigo was already maximal on the fourth floor. Thus subjective height vertigo seems to be saturated and maximal at a height of about 20 m. Habituation may have contaminated the data, however. This is suggested by the slight decrease of the mean scaling value at floor 10 compared with the standard at floor 4.

The stabilograms of postural sway taken under these natural environmental conditions were affected by occasional wind and an extensive body tremor due to acrophobia (Fig. 6). This tremor had a frequency of 5-10 Hz and did not represent the visually induced sway of height vertigo because it continued



## SUBJECTIVE HEIGHT VERTIGO

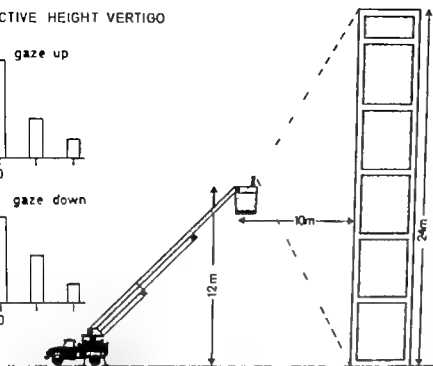
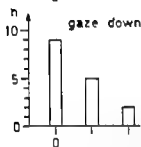
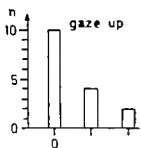


Fig 4 Relative influence of downward versus upward gaze on occurrence of subjective height vertigo by use of the open cabin of a fire-brigade ladder in front of a 24-metre tower. Scalings (0 = none, + = moderate, ++ = medium) of 16 volunteer subjects revealed. Height vertigo can be induced with gaze up. Distance rather than the direction of gaze appears to be most critical.

dium (++) and severe (+++) height vertigo. The subjects demonstrated no problems in understanding and performing this task.

As seen in Fig 3 maximal height vertigo occurred with upright stance where most of the subjects reported severe (8) or medium (7) vertigo. Only two persons felt free of any distressing effects while standing at the edge. Sitting although objectively quite dangerous was tolerated much better. Only three had severe and six medium vertigo. The elbow-knee position was even less disturbing with no subject having severe and only 4 medium vertigo. Finally the lying position was best tolerated and did not evoke height vertigo symptoms in the majority of the subjects. Thus the magnitude of subjective height vertigo obviously depends upon body position and is the greatest with upright stance.

#### Experiment II Influence of downward versus upward gaze

Sixteen voluntary subjects (9 male, 7 female, average age 28) were blindfolded and separately taken to an altitude of 12 m in the small open cabin of a fire-brigade ladder in front of a 24 m tower (Fig 4). Upon the experimenter's command the subjects looked at either the

upper or lower end of the tower. Peripheral vision was restricted to an angle of about 120 degrees by use of a funnel attached to the head. This was to exclude possible cues from the cabin's contrasts. There were no moving objects such as clouds which might complicate the experiment by inducing postural instability through optokinetic linearvection. Eight subjects started with gaze up and 8 with gaze down. As in experiment I scaling was none (-), moderate (+), medium (++) or severe (+++) height vertigo. 5s were mainly height vertigo resistant because knowledge of the stimulus conditions served to weed out the fearful candidates. This subject selection process and the low altitude probably were responsible for the low levels of the scaling responses. There was no significant difference for gaze-down as compared with the gaze-up conditions (Fig 4). Thus subjective height vertigo seems to be independent of gaze direction and dependent only on the distance between subject and visual scene.

#### Experiment III Influence of altitude

Fifteen volunteer subjects (7 male, 8 female, average age 32) participated in this experiment on a high building under construction. Un-

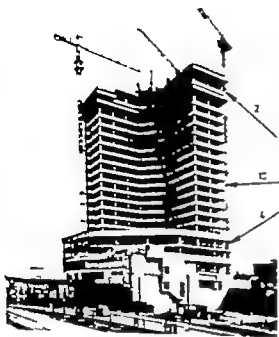


Fig. 5. Saturation of subjective height vertigo at increasing altitudes (20, 50 and 100 metres, 4th, 10th, and 16th floor respectively). Magnitude estimations of 15 subjects

are not significantly different from each other (p > 0.05) (Mann-Whitney U-test).

desired perceptual cues provided by windows and visible ceilings were prevented by restriction of peripheral vision to a total field of 120 degrees in diameter. Subjects were blindfolded and taken in an outside elevator to the fourth floor at an altitude of approximately 20 m. Thereupon via a staircase they stepped onto the stabilometer platform which stood adjacent to the outer wall of the building. The blindfolds were removed and the subjects suddenly exposed to the height vertigo inducing situation during which they were required to look down for a period of one minute. A security belt was used as a precautionary measure. The fourth floor condition served as the standard situation for further magnitude estimations of subjective vertigo (modules with an arbitrary value of 10). Posturographic measurements were performed of the fore-aft and lateral body sway with eyes closed and eyes open as well as obtaining the subject's scalings of height vertigo severity. All's were then taken from the fourth to the twentieth and finally to the tenth floor representative of

the range of altitudes investigated. Technical factors such as the limited number of subjects and the limited number of measurements per subject were considered.

As expected, the magnitude of height vertigo increased with increasing altitude. The magnitude of height vertigo was significantly greater at the fourth, tenth and twentieth floors than at the ground level. The magnitude of height vertigo was significantly greater at the twentieth floor than at the fourth and tenth floors. The magnitude of height vertigo was significantly greater at the fourth floor than at the ground level.

The subjects' ratings of height vertigo severity were significantly greater at the twentieth floor than at the fourth and tenth floors. The subjects' ratings of height vertigo severity were significantly greater at the fourth floor than at the ground level.



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## SUBJECTIVE HEIGHT VERTIGO

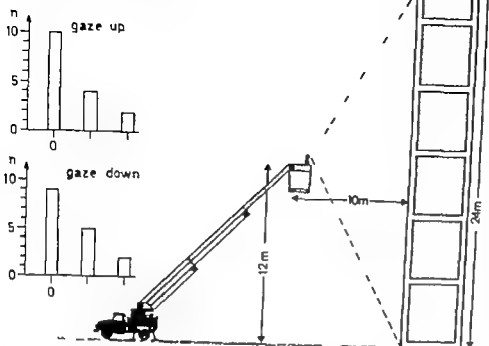


Fig. 4 Relative influence of downward versus upward gaze on occurrence of subjective height vertigo by use of the open cabin of a fire brigade ladder in front of a 24 metre tower. Scalings (0 = none, + = moderate, ++ = medium, +++ = severe) of 16 volunteer subjects revealed. Height vertigo can be induced in gaze up. Distance rather than the direction of gaze appears to be most critical.

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vertigo does not occur on the ground despite the fact that visual targets may be at a great distance because cues from nearby stationary contrasts (provided by peripheral vision) prevent the instigating sensory mismatch.

This might become different if the ground is not aligned to the earth horizontal although the relative influence of the angle of incline/decline has not been studied thoroughly. In preliminary experiments we found (with great intra- and inter-subject variability) that for a given altitude subjective height vertigo can be induced at an angle of decline of 45–50 degrees and that it progressively increases with increasing slope up to a maximal vertigo. With increasing slope peripheral vision provides less information about nearby stationary contours. According to posturographic measurements the development of subjective height vertigo also requires an exposure time of 5 to 30 sec to be saturated.

All visual stimulus characteristics which have an influence on the occurrence and intensity of physiological height vertigo concomitantly affect postural balance. Purkinje (1820) was the first to attribute height vertigo symptoms to a postural imbalance of upright station (he stressed the unusual and therefore unadapted stimulation at gaze downward with the stationary visual reference in an imaginary space below foot support) as later was assumed more concretely by Lee & Lishmann (1975), Brandt (1976), Bles et al (1978) and Brandt et al (1978). Purkinje (1820) and Kobrak (1974) erroneously concluded as have modern textbooks that height vertigo mainly represented a disorder in neurasthenic or psychiatric patients.

Pogány's (1958) finding, that patients with vestibular dysfunction exhibited a greater susceptibility to height vertigo, is now understandable since the erroneous visual signal should have a disproportionately greater sensorial weight when associated with a vestibular lesion. This is analogous to our experiments (paper II Bles et al 1979) where induced somatosensory or vestibular systems

disturbances (e.g. when standing on foam rubber or being carried in a quivering open elevator attached to the outside wall of a building under construction) enhanced subjective height vertigo symptoms.

### *Physical prevention of height vertigo*

Knowledge of the stimulus characteristics required for the optimal visual contribution to stabilized postural balance also dictates practical advice related to the reduction of height vertigo in susceptible subjects.

1. One should avoid the free upright stance in critical situations at high altitudes. This is done intuitively when grasping for stationary framework or leaning against a wall for support.

2. When looking down, one should obtain stationary cues from nearby contrasts in the peripheral visual field. (Visual stabilization of posture is served primarily by the retinal periphery while the central retina mainly serves egocentric recognition and pursuit of objects.) Staring at moving objects such as clouds increases the danger of falling because additional postural destabilization through linearvection may be induced. One should avoid long exposure times as height vertigo usually has a latency taking several seconds to develop. Looking through binoculars is very dangerous because it restricts the visual field and introduces the unusual and therefore unadapted magnification factor.

3. Body and head position should be adjusted to the gravitational vector because vision will receive a relatively greater sensorial weight (which is undesirable) if the otoliths are displaced beyond their optimal working range by extreme head tilt. It may also be true on the basis of other observations that the feet should be firmly planted on an 'earth horizontal' surface.

### *Physiological height vertigo versus acrophobia*

Physiological height vertigo must be differentiated from neurotic acrophobia or height

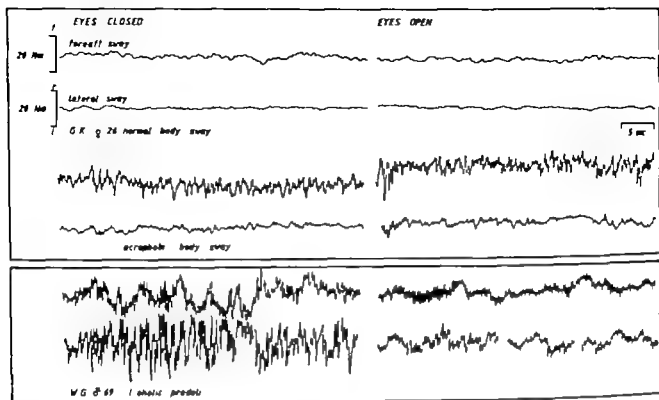


Fig 6 Fore-aft and lateral body sway (stabilogram) of an acrobatic subject on ground as compared with a height vertigo inducing situation on a high building. Expectation of exposure to high altitude with eyes closed already causes a postural destabilization and body tremor

This tremor whose frequency is 5–10 Hz, is similar to the body tremor of patients suffering from alcoholic predebr but is not related to the low frequency destabilization of physiological height vertigo

with eyes closed. Two S's became extremely fearful and the experiments had to be interrupted. In one subject the security belt was needed to prevent a catastrophic fall.

We tested two tight rope artists under the same conditions. Although they did not admit to subjective height vertigo, they were not willing to perform the experiments without security belts. The artists' stabilograms exhibited a sway with the same characteristics found in the untrained subjects under identical conditions. Paper II will provide posturographic evidence of the body sway amplitudes of normals being dependent on eye-object distance.

### COMMENT

#### *Height vertigo: a distance vertigo*

The psychophysical and posturographic data obtained under natural height vertigo conditions were consistent with our hypothesis that

height vertigo was due to a visual destabilization of posture when the distance between the eyes and stationary contrasts in the surround becomes critically large. The significant increase in body sway amplitudes (paper II, Bles et al 1979) reflects a postural imbalance which introduces a real danger of falling from the high position.

Our trigonometrical model based on an assumed retinal movement detection threshold of about 20 min of arc predicted that height vertigo would be the most pronounced in the upright stance, that it would increase with increasing eye-object distance with a saturation below 15–20 m net altitude, and that upward and downward gaze should induce similar responses. All of these predictions were confirmed by the scaling experiments. Physiological height vertigo appears to be really a distance vertigo because the eye-object distance and not the net altitude or the visual direction is the critical stimulus. Distance ver-

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anxiety in which the altitude acts only as the inducing stimulus situation for a pathological phobic reaction. Acrophobia may even become symptomatic on the ground during imagination of high altitudes or if one is exposed to stereographic slides of a downward view from high buildings (Takeya et al 1979). Goethe (1771) was probably the first to report on repeated exposure to height as a valuable therapy of acrophobia which he experienced on top of the Strassburger Münster. This later was developed into a behavioral therapy concept of self directed contact desensitization (Ritter 1969; Baker et al 1973) or implosion therapy (flooding) after Marks & Gelder (1967). It is obvious from our experiments that these therapies are concerned with the psychiatric aspects of acrophobia but do not affect physiological height vertigo as described above.

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### ZUSAMMENFASSUNG

Der physiologische Höhenwindel ist eher ein „Distanzwindel“ hervorgerufen durch visuelle Destabilisierung der aufrechten Haltung, wenn der Abstand zwischen Auge und den nächsten stationären Kontrasten im Gesichtsfeld eine kritische Größe erreicht. Obwohl der Höhenwindel allgemein als angstneurotisches Syndrom angesehen wird, gibt es eine geometrische Erklärung für eine physiologische Genese: ein intensitätsmischer Konflikt der Wahrnehmung von Körperbewegungen. Vestibuläre und propriozeptive Meldungen widersprechen den visuellen Informationen.

Psychophysische Daten bestätigen die Hypothese: 1) Höhenwindel hängt von der Körperhaltung ab und ist beim freien Stand am stärksten; 2) Nicht die absolute Höhe sondern die Auge-Objekt-Distanz ist entscheidend; 3) Mit zunehmender Höhe nimmt der Windel zu, ist jedoch bei 20 Metern bereits gesättigt.

Physiologischer „Distanzwindel“ sollte von der psychopathologischen Acrophobie unterschieden werden. Die physiologische Haltungsinstabilität unter Höhenwindelbedingungen kann verhindert werden, wenn man darauf achtet, nahe stationäre Kontraste im peripheren Gesichtsfeld zu behalten.

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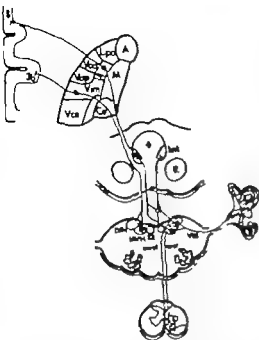


Fig. 1. Diagram showing the main ascending and descending efferents. The denomination of the brain nuclei corresponds to Hensley's nomenclature (LVN and MVN: lateral and medial vestibular nuclei; MVST and LVST: medial and lateral vestibulo-spinal tracts).

with to the different parts of the vestibular nuclear complex. From Lorente de Nó's observations (1938) we know that semicircular ducts project chiefly to the superior and medial nuclei, whereas the utricle supplies the lateral nucleus (or Donders nucleus) (Fig. 1).

The specific stimulus for the semicircular canals consists in angular acceleration or deceleration of the head which activates the ampullar cristae during movements of the head. The result of this is the nystagmic reaction characterized by slow conjugated deviation of the eyes in the plane of the canal and counter to the direction of movement (slow phase) with a rapid return to the previous position (fast phase). Thus the semicircular canals that Brodal calls kinetic labyrinth records angular movements of the head. This is in agreement with the anatomical data, since the medial vestibular nucleus that receives the

impulses from the ampullar cristae projects in turn to the oculomotor nuclei (via the fasciculus longitudinalis medialis) and to the cervical and upper thoracic spinal cord (via the medial vestibulo-spinal tract).

On the other hand the specific stimulus for the hair cells of the utricular macula is the pressure of the otoliths over the particular region where they rest according to the varying attitudes of the head. The utricle—and probably the saccule too—constitute the static or tonic labyrinth. Its impulses are transmitted mainly to the lateral vestibular nucleus of Donders and thence to the lumbar and sacral spinal cord (via the lateral vestibulo-spinal tract).

The lateral vestibulo-spinal tract terminates entirely according to histological studies of Nyberg-Hansen in the ipsilateral laminae VII and VIII of Rexed of the lumbosacral spinal cord. Laminae VII and VIII contain exclusively the soma of interneuron that of motoneuron being located in lamina IX. However the dendrites of the latter extend to far distant points in laminae VII and VIII (Ramon y Cajal, L. de Nô, Scheibel & Scheibel). There is thus histological data suggesting monosynaptic axodendritic connections between vestibulo-spinal fibres and motoneurons as well as axo-somatic contact with interneurons. Since the work of Lund & Pompeiano in 1965 various authors have provided further physiological evidence in support of this view. From these studies it appears that the descending lateral vestibulo-spinal fibre ends monosynaptically in  $\alpha$ -motoneurons (Grillner et al., Shapovalov, Sherrington)  $\gamma$ -motoneurons (Grillner et al., Carli et al., Pompeiano et al.) and interneurons (Grillner et al.). Grillner in 1972 succeeded in provoking monosynaptic EPSPs in ipsilateral  $\alpha$  and  $\gamma$ -motoneurons of extensor nucleus of the ankle following stimulation of Donders nucleus. Only occasionally can monosynaptic EPSPs be recorded in ipsilateral extensor of the knee. Indirect evidence suggests that their effects are exerted almost exclusively on static  $\gamma$ -motoneurons (S. Grillner). Disynaptic



# THE ROLE OF THE VESTIBULAR SYSTEM IN RELATION TO MUSCLE TONE, AND POSTURAL REFLEXES IN MAN

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**Abstract** The LVST in man seems to provoke through its activation from the utricle in response to displacement of the center of gravity an increase of the excitability of  $\gamma$ -static motoneurons of extensor muscles of trunk and legs and in a lesser degree of  $\gamma$ -dynamic and  $\alpha$ -motoneurons. This contributes to the adjustment of muscle tone for the maintenance of the static standing posture and for the alternative change from the extensor to the flexor phase during locomotion. Temporary vestibular hyperfunction provoked by central or peripheral stimulation is manifested clinically by vertigo. There is during this state a hyperexcitability of  $\gamma$ -static motoneurons with lesser or no change of  $\gamma$ -dynamic and  $\alpha$ -motoneurons. Suppression of vestibular function by destruction of the labyrinth section of the vestibular nerve interruption of the central vestibular pathways or of the LVST result in disequilibrium with loss of postural control. A marked depression of  $\gamma$ -static innervation can be detected in this case. The presence of adequate  $\gamma$ -static innervation in one side seems to be contradictory to complete suppression of labyrinthine function in this same side. On the other hand absence of response to caloric stimulation in one or both labyrinths does not exclude the possibility of vestibular hyperfunction. The study of phasic stretch reflexes appears to be a valid method of measurement of the functional state of the static labyrinth and of the LVST.

The role of the labyrinth and of the vestibular nucleus in postural reactions was demonstrated by Sherrington in his remarkable experiments upon decerebrate animals. Transection of the brain stem at an intercollicular level provokes in the animal a permanent postural change whose description by Sherrington (1898) is still classic: the animal then in absence of external stimulation remains with extended limbs and neck whether set on its side or prone or supine. If placed upright upon its feet it retains the position so given it. The weight of the various parts is then supported by the continuous contractions of muscles

counteracting gravity. Sherrington demonstrated that upright stance is maintained by the permanent contraction of antigravity muscles which he called *stretch reflex*.

Later he distinguished this *tonic stretch reflex* from the brief contraction of extensor muscles to sudden stretch which he named *phasic stretch reflex* nowadays called *tendon jerks*. Sherrington further demonstrated that labyrinthine influences maintain the decerebrate rigidity following sectioning of the vestibular nerve destruction of the nucleus of Deiters or sectioning of the lateral vestibulospinal tract (LVST) suppress decerebrate rigidity. In 1906 Sherrington wrote each labyrinth maintains tonus especially in the neck and trunk muscles and in the extensor abductor limb muscles of the homonymous side."

Since both the tonic and the phasic reactions are a manifestation of the same myotatic or stretch reflex its modification in health or in disease is always parallel. Hence analysis of tendon jerks much more easily reproducible and measurable than the tonic stretch reflex provides an index of the functional state of postural reactions. Alteration of labyrinthine function will induce changes in postural reactions that can be detected and measured by analysis of tendon jerks.

## Anatomical and Physiological Studies on Vestibulo-spinal Projections

There is a precise topographical arrangement of the projections from each part of the laby

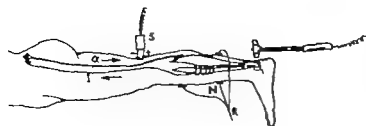


Fig 3 The position of the recording and stimulating electrodes as well as the pathways involved in the myotatic reflex are represented

the ankle joint is passively relaxed this minimum degree of  $\gamma$  static innervation due to the force of gravity will further diminish and the response thus obtained (which we call AI (Fig. 4)) will be proportionately reduced. In a normal individual the ratio AI/AR indicates the proportion of  $\gamma$ -static versus  $\gamma$ -dynamic innervation whereas the index AI/M will indicate the proportion of  $\gamma$ -static versus the total pool of motoneurons. An increase or decrease in LVST activity will respectively increase or decrease the value of AI/AR and AI/M.

The Jendrassik manoeuvre has been shown to provoke an increase in facilitatory suprasegmentary influences primarily of  $\gamma$ -dynamic and static motoneurons (J. Paillard). AI and AR are effectively increased during the manoeuvre in normal subjects. The degree of facilitation is inversely proportional to the degree of excitability of motoneurons. Thus in spastic patients in which the loop is already saturated the effectiveness of the Jendrassik manoeuvre to increase AI—and still more the AR—is reduced or even abolished. Inversely desaturation of the  $\gamma$  loop by a lack of activity of facilitatory influences will relatively increase the effectiveness.

The excitability of  $\alpha$ -motoneurons is measured by the index H/M and by the recovery curve of the H reflex (J. W. Maglander). Passive relaxation of the ankle joint or the Jendrassik manoeuvre induces very little if any modification of the value of H and did not modify the duration of the refractory periods of motoneurons at all (N. Porrier et al.). Increase in  $\alpha$ -motoneuron excitability is mani-

fested by an increase in H/M and a shortened refractory period. Conversely a reduction in the  $\alpha$ -motoneuron excitability induces a fall in H/M and a lengthening of the refractory period.

## MATERIAL

In order to determine the function of the LVST in man we compared the values of stretch reflexes in 54 healthy persons with those of patients with hyper and hypofunction of the vestibular system. The former group

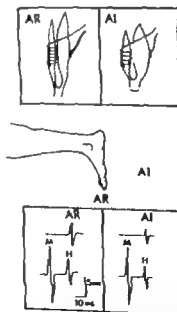


Fig 4 With the gastrocnemius in a completely relaxed position the mechanically induced reflex AI is smaller than the reflex obtained with the foot in a natural resting position called AR. The H reflex and the M response have the same value in both instances.

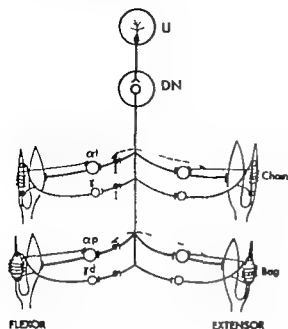


Fig. 2 The main connections of the LVST with spinal motoneurons are presented (U utriculus DN Dorsal nucleus  $\alpha$  alpha tonic motoneurons  $\alpha p$  alpha phasic motoneurons  $\gamma s$  gamma static motoneurons  $\gamma d$  gamma dynamic motoneurons)

EPSP can also be provoked in  $\alpha$  motoneurons of ipsilateral extensor of the ankle and in a group of flexor of the toe. Disynaptic IPSPs can also be evoked in ipsilateral  $\alpha$  and  $\gamma$ -motoneurons of knee and ankle flexors as well as in a small group of hip extensors.

Disynaptic EPSPs can be evoked in contralateral hip, knee and ankle extensors through ipsilateral interneurons. Finally, trisynaptic IPSP can be evoked in contralateral flexor motoneurons (Grillner & Hongo).

In relation to the effect on the spinal reflex pathways Grillner remarks that the vestibulo-spinal tract facilitated generally reflex areas which give the same effect to  $\alpha$  motoneurons as it gives itself, i.e. *excitation of extensors and inhibition of flexors*. The effects are exerted bilaterally.

Fig. 2 summarizes the previous experiences.

From these experiences it appears—according to Germandt (1967)—that the fact that two antagonistic spinal reflex responses the extensor gastrocnemius and the flexor tibialis anticus are facilitated suggests that vestibular (LVST) influences at the spinal seg-

mental level contribute to co-contractional muscular patterns necessary to the pillar-like stability of the weight-bearing limb (B Germandt). On the other hand Grillner & Hongo (1972) concluded that the function of the (lateral) vestibulo-spinal tract during locomotion is to coordinate and adjust the extensor activity to an optimal level during each cycle presumably through the influence of cerebellum or its nuclei (Grillner & Hongo).

## METHOD

The stretch reflex has a phasic and a tonic component. The phasic reflex can be elicited mechanically by a sudden tap over the tendon. This is the case of tendon jerks (Fig. 3). The stretching of a muscle spindle monosynaptically excites the  $\alpha$ -motoneurons innervating the same muscle which will immediately contract. This reflex (A) and its modification by various manoeuvres can be easily recorded and measured by applying the technique described by Paillard. On the other hand due to the fact that the threshold of Ia fibres is lower than that of the motor axons it is possible to provoke a phasic stretch reflex by electrical stimulation of the former beneath the muscle spindles. The reflex thus obtained is called (according to Hoffman) the H reflex (Fig. 3).

The amplitude of A depends mainly on the state of excitability (readiness to fire) of the muscle spindles conditioned in turn by excitatory and inhibitory suprasegmentary impulses. The more important of the former is precisely the LVST. Its action induces an increase in tension of nuclear chain intrafusal fibres through activation of static  $\gamma$  motoneurons. On the other hand due to the  $\alpha$ - $\gamma$  linkage the amplitude of A will also be in relation with the degree of excitability of  $\alpha$  motoneurons. The tendon jerk obtained in a resting position will be called AR (Fig. 4). This value depends on the degree of excitability of  $\alpha$ -phasic and  $\alpha$ -dynamic motoneurons at rest added to the degree of excitability of  $\gamma$ -static motoneurons in a natural resting position. If

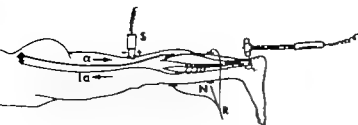


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## MATERIAL

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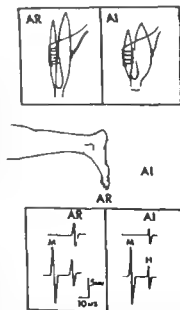


Fig. 4 With the gastrocnemius in a completely relaxed position the mechanically induced reflex AI is smaller than the reflex obtained with the foot in a natural resting position called AR. The H reflex and the M response have the same value in both instances.

Table 1 *Modification of contralateral of stretch reflexes following stimulation of central vestibular pathways (2 cases)*

|                    | H reflex     | H/M Index        | AI            | AR            | AI/AR          | AI+J         | AR+J        | AI/H          | AI/M         | AI/J          | AI |
|--------------------|--------------|------------------|---------------|---------------|----------------|--------------|-------------|---------------|--------------|---------------|----|
| At rest            | 7            | 36%<br>M 19 mlvs | 3             | 5             | 60%            | 5<br>(+66%)  | 8<br>(+60%) | 48%           | 15           | 79            | 3  |
| During stimulation | 11<br>(+46%) | 57%<br>(+15%)    | 6<br>(+100%)  | 10<br>(+100%) | 60%            | 8<br>(+33%)  | 1<br>(+70%) | 54%<br>(+11%) | 31<br>(+16%) | 90<br>(+11%)  | 1  |
| After lesion       | 7<br>(=)     | 36%<br>(=)       | 0.5<br>(-84%) | 2<br>(-46%)   | 2.2%<br>(-38%) | 1<br>(+100%) | 4<br>(+81%) | 7%<br>(-75%)  | 2%<br>(-13%) | 31%<br>(-40%) | 1  |

comprised 2 patients where electrical stimulation of the central vestibular pathways was performed and one patient who underwent caloric stimulation. The latter group was integrated with 18 patients treated surgically by vestibular neurectomy: 9 cases of antero-lateral cordotomy and 2 patients with surgically induced lesions of central vestibular pathways. In all the patients selected we have well documented pre- and postoperative recordings of myotatic reflexes.

Finally, once the electrophysiological criteria of hyper- or hypofunction of the vestibular system were established, the study of stretch reflexes was extended to an unselected group of patients suffering from various types of vestibular dysfunction.

## RESULTS

### *Group 1: Temporary vestibular hyperfunction during electrical stimulation of central vestibular pathways*

During stereotactic procedures at the level of the thalamus and basal ganglia for the control of pain, abnormal movements and epilepsy, the stimulating electrode is occasionally placed at the level of the tegmental dorso-lateral bundle, known in the classical literature by the denomination secondary dorsal trigeminal tract of Wallemberg. The vestibular nature of this pathway was demonstrated by Hess, who described ipsiversive torsion of the feline head during its stimulation. Hassler

further demonstrated that this bundle terminates in the neurons of the ventral part of the Deters nucleus and ends in the nucleus ventro-intermedius (Vim) of the thalamus. Low intensity and high frequency stimulation at this level provokes rotatory vertigo and various other postural hallucinations. The study of stretch reflexes during the stimulation (Table 1) shows a marked temporary increase in excitability of  $\alpha$ -static and  $\gamma$ -dynamic neurons and a moderate increase of neurons excitability. This effect is more pronounced in the contralateral side.

Interruption of this pathway by stereotactic surgery is followed by cor-hypotonia and loss of postural control. A marked diminution of  $\gamma$ -static innervation can be demonstrated by the depression of AI and AI/M. A marked reduction of  $\gamma$ -dynamic innervation is testified by a less significant reduction of AR (Table 1). Since the H-reflex and of the H/M index changed, the excitability of  $\alpha$ -static seems to be less influenced by the central vestibular pathways. The test became positive after surgery remained so for months afterwards. The diminution in stretch reflexes follows a parallel with that of the muscle tone. Years later when adaptation is complete, the clinical point of view, deep static innervation is still detectable.

Table II Modification of ipsilateral stretch reflexes following caloric ( $0^{\circ}$ ) stimulation (1 case)

|                     | H reflex | H/M                | AI   | AR   | AI/AR | AI+J         | AR+J        | AI/H | AI/M | AR/H | AR/M |
|---------------------|----------|--------------------|------|------|-------|--------------|-------------|------|------|------|------|
| Pre                 | 5        | 31%<br>M. 16<br>mV |      | 5    | 40%   | 4<br>(+100%) | 8<br>(+60%) | 40%  | 12%  | 100% | 31%  |
| Post<br>stimulation | 6        | 37%                | 4    | 8    | 50%   | 5<br>(+25%)  | 8<br>(10)   | 66%  | 25%  | 133% | 90%  |
| Deviance            |          | +6%                | 100% | +60% | 10%   | 75%          | -100%       | +26% | +13% | +33% | +25% |

### Group 2 Temporary vestibular hyperfunction using caloric ( $0^{\circ}$ ) stimulation

This experiment was performed in a single case of benign positional vertigo. With the patient lying on his back, the stretch reflexes are provoked with mechanical and electrical stimulation. A few seconds after commencing irrigation a marked increase in AI and a moderate increase in AR were detected (Table I). The effect was bilateral but more pronounced ipsilaterally. Since the values of AI and AR are increased the effectiveness of the Jendrassik's manoeuvre is proportionally reduced. Caloric activation of the labyrinth could therefore seem to enhance the excitability of  $\gamma$ -static and to a lesser degree,  $\gamma$ -dynamic motoneurons.

### Group 3 Modification of stretch reflexes following surgical lesion of the vestibular nerve

Fourteen patients with various vestibular pathologies were selected in whom pre and post-operative studies of myotatic reflexes were performed. The results (Table III) correspond exclusively to ipsilateral reflexes obtained within 7 weeks after surgery. From the clinical standpoint all patients presented a marked abnormal Romberg test that persisted although progressively attenuated for 17 to 18 months. But even after 7 years with the patient's feet placed in front of one another and his eyes closed, the Romberg test is still abnormal when the foot on the side of the neurectomy is behind the other.

Immediately after surgery there is a marked fall in the value of AI and a much more moderate decrease in AR and H (Table III). Since Jendrassik's manoeuvre is still strongly positive for AI after surgery it appears that the suppression of vestibular function provokes a selective diminution of the excitability of  $\gamma$ -static motoneurons. Repeated studies after periods of up to 5 years following surgery

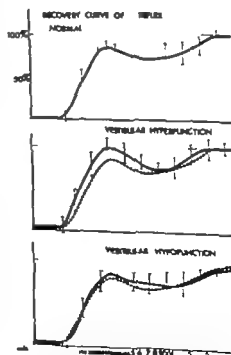


Fig. 5 Recovery curve of the H reflex in normal persons and in patients with hyper and hypofunction of the vestibular system. The dotted lines in the second and third curve correspond to the average normal result.

Table 1 *Modification of contralateral of stretch reflexes following stimulation and lesion of central vestibular pathways (2 cases)*

|                    | H reflex     | H/M index           | AI            | AR            | AI/AR         | AI+J         | AR+J         | AI/H         | AI/M          | AR/H          | AR/M          |
|--------------------|--------------|---------------------|---------------|---------------|---------------|--------------|--------------|--------------|---------------|---------------|---------------|
| At rest            | 7            | 36%<br>M 19<br>mlvs | 3             | 5             | 60%           | 5<br>(+66%)  | 8<br>(+60%)  | 48%          | 15%           | 71%           | 26%           |
| During stimulation | 11<br>(+56%) | 57%<br>(+71%)       | 6<br>(+100%)  | 10<br>(+100%) | 60%           | 8<br>(+33%)  | 17<br>(+70%) | 54%<br>(+1%) | 31%<br>(+16%) | 90%<br>(+21%) | 52%<br>(+26%) |
| After lesion       | 7<br>(=)     | 36%<br>(=)          | 0.5<br>(-84%) | 2.2<br>(-56%) | 22%<br>(-38%) | 1<br>(+100%) | 4<br>(+81%)  | 7%<br>(-35%) | 2%<br>(-13%)  | 31%<br>(-40%) | 11%<br>(-15%) |

comprised 2 patients where electrical stimulation of the central vestibular pathways was performed and one patient who underwent caloric stimulation. The later group was integrated with 18 patients treated surgically by vestibular neurectomy, 9 cases of antero-lateral cordotomy and 2 patients with surgically induced lesions of central vestibular pathways. In all the patients selected we have well documented pre, per and postoperative recordings of myotatic reflexes.

Finally, once the electrophysiological criteria of hyper or hypofunction of the vestibular system were established, the study of stretch reflexes was extended to an unselected group of patients suffering from various types of vestibular dysfunction.

## RESULTS

### *Group 1: Temporary vestibular hyperfunction during electrical stimulation of central vestibular pathways*

During stereotactic procedures at the level of the thalamus and basal ganglia for the control of pain, abnormal movements and epilepsy, the stimulating electrode is occasionally placed at the level of the tegmental dorso-lateral bundle, known in the classical literature by the denomination secondary dorsal trigeminal tract of Wallemberg. The vestibular nature of this pathway was demonstrated by Hess, who described ipsiversive torsion of the feline head during its stimulation. Hassler

further demonstrated that this bundle originates in the neurons of the ventral part of the Deiters nucleus and ends in the nucleus ventro-intermedius (Vim) of the thalamus. Low intensity and high frequency stimulation at this level provokes rotatory vertigo and various other postural hallucinations. The study of stretch reflexes during the stimulation (Table 1) shows a marked temporary increase in excitability of  $\alpha$ -static and  $\gamma$ -dynamic motoneurons and a moderate increase of  $\alpha$ -motoneurons excitability. This effect is bilateral but more pronounced in the contralateral side.

Interruption of this pathway by opening with the fine blunt wire employed in stereotactic surgery is followed by contralateral hypotonia and loss of postural control in the leg. A marked diminution of  $\gamma$ -static innervation can be demonstrated by the pronounced depression of AI and AI/M. A moderate reduction of  $\gamma$ -dynamic innervation is manifested by a less significant reduction in the value of AR (Table 1). Since the values of the H reflex and of the H/M index remain unchanged, the excitability of  $\alpha$ -motoneurons seems to be less influenced by the lesion of the central vestibular pathways. Romberg's test became positive after surgery and remained so for months afterwards. The alteration in stretch reflexes follows a course parallel with that of the muscle tone. But even 10 years later when adaptation is perfect from the clinical point of view, depression of  $\gamma$ -static innervation is still detectable.

Table II Modification of ipsilateral stretch reflexes following caloric ( $0^{\circ}$ ) stimulation (1 case)

|                    | H reflex | H/M                 | AI    | AR   | AI/AR | AI+J         | AR+J        | AI/H | AI/M | AR/H | AR/M |
|--------------------|----------|---------------------|-------|------|-------|--------------|-------------|------|------|------|------|
| 1st                | 5        | 31%<br>ML 16<br>mVs |       | 5    | 40%   | 4<br>(+100%) | 8<br>(+60%) | 40%  | 12%  | 100% | 31%  |
| During stimulation | 6        | 37%                 | 4     | 8    | 50%   | 5<br>(+25%)  | 8<br>(0)    | 66%  | 25%  | 133% | 50%  |
| Recovery           | 20%      | 6%                  | +100% | +60% | +10%  | -75%         | -100%       | +76% | +13% | +33% | +25% |

### Group 2 Temporary vestibular hyperfunction during caloric ( $0^{\circ}$ ) stimulation

his experiment was performed in a single case of benign positional vertigo. With the patient lying on his back, the stretch reflexes are provoked with mechanical and electrical stimulation. A few seconds after commencing stimulation a marked increase in AI and a moderate increase in AR were detected (Table I). The effect was bilateral but more pronounced ipsilaterally. Since the values of AI and AR are increased, the effectiveness of the Jendrassik's manoeuvre is proportionally reduced. Caloric activation of the labyrinth could therefore seem to enhance the excitability of  $\gamma$ -static and in a lesser degree  $\gamma$ -dynamic motoneurons.

### Group 3 Modification of stretch reflexes following surgical lesion of the vestibular nerve

Eighteen patients with various vestibular pathologies were selected in whom pre and post-operative studies of myotatic reflexes were performed. The results (Table III) correspond exclusively to ipsilateral reflexes obtained within 7 weeks after surgery. From the clinical standpoint all patients presented a marked abnormal Romberg test that persisted although progressively attenuated for 1 to 18 months. But even after 7 years with the patient's feet placed in front of one another and his eyes closed the Romberg test is still abnormal when the foot on the side of the neurectomy is behind the other.

Immediately after surgery there is a marked fall in the value of AI and a much more moderate decrease in AR and H (Table III). Since Jendrassik's manoeuvre is still strongly positive for AI after surgery it appears that the suppression of vestibular function provokes a selective diminution of the excitability of  $\gamma$ -static motoneurons. Repeated studies after periods of up to 5 years following surgery

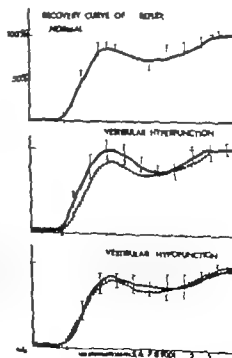


Fig. 5 Recovery curve of the H reflex in normal period and in patients with hyper and hypofunction of the vestibular system. The dotted lines in the second and the curve correspond to the average normal result.



Table III *Modification of ipsilateral stretch reflexes following vestibular neurectomy (18 cases)*

|            | H    | H/M                   | AI   | AR  | AI/AR | AI+J           | AR+J        | AI/H | AI/M | AR/H | AR/M |
|------------|------|-----------------------|------|-----|-------|----------------|-------------|------|------|------|------|
| Before     | 8.3  | 48%<br>M 17.1<br>mlvs | 4    | 6   | 66%   | 6.4<br>(+60%)  | 7<br>(+16%) | 48%  | 23%  | 77%  | 35%  |
| After      | 6.8  | 39%                   | 2.1  | 5.5 | 38%   | 4.2<br>(+100%) | 6<br>(+9%)  | 30%  | 12%  | 80%  | 1%   |
| Difference | -18% | -9%                   | -48% | -9% | -28%  | -40%           | -7%         | -18% | -11% | +1%  | -3%  |

show a progressive attenuation of this effect. It is our opinion that if the myotatic reflexes are normal before surgery and the surgical suppression both of the vestibular nerve and of the ganglion of Scarpa are complete, the decrease in  $\gamma$  static innervation will remain definitive. Should a complete normalization of reflexes occur after surgery, the possibility of an incomplete ablation must be considered. This possibility is still greater if there is recurrence of vertigo.

#### Group 4. *Modification of stretch reflexes after antero-lateral cordotomy*

The LVST is situated immediately in front and slightly medial to the lateral spino-thalamic tract. Sectioning of the latter for relief of pain is therefore accompanied by interruption of the former. Hypotonia and loss of postural control in the ipsilateral leg always occur following antero-lateral cordotomy. The paresis that occasionally occurs when a too posterior lesion encroaches on the lateral cortico spinal tract is also accompanied by immediate hypo-

tonia and imbalance, but the characteristic hyperreflexia and spasticity of the pyramidal syndrome will appear shortly after.

The study of stretch reflexes following antero-lateral cordotomy shows the most dramatic and selective loss of  $\gamma$ -static innervation (Table IV). Indeed for a period of about 7 to 4 months, the ankle jerk in the maximal relaxation position (AI) remains absent, whereas the diminution of the ankle jerk in a resting position (AR) is minimal. The excitability of  $\alpha$ -motoneurons is not affected significantly by transection of the LVST.

## DISCUSSION

From the results presented it appears that vestibular hyperfunction provoked by caloric stimulation of the labyrinth or by electric stimulation of central vestibular pathways is accompanied clinically by various postural hallucinations and primarily by vertigo. The study of stretch reflexes in these states demonstrates the hyperexcitability of extensor

Table IV *Modification of ipsilateral stretch reflexes following anterolateral cordotomy (9 cases)*

|            | H reflex               | H/M | AI    | AR   | AI/AR | AI+J        | AR+J        | AI/H  | AI/M  | AR/H | AR/M |
|------------|------------------------|-----|-------|------|-------|-------------|-------------|-------|-------|------|------|
| Before     | 6.5 ml<br>M 19<br>mlvs | 34% | 2.5   | 4.5  | 55%   | 4<br>(+60%) | 7<br>(+55%) | 13%   | 38%   | 3%   | 69%  |
| After      | 6                      | 31% | 0     | 3    | 0%    | 0           | 4<br>(+5%)  | 0     | 0     | 15%  | 50%  |
| Difference | 0                      | 0   | -100% | -33% | -100% | 0           | -43%        | -100% | -100% | 35%  | -28% |

Table V Comparison between stretch reflexes in normal (54) vestibular hyperfunction (13) and vestibular hypofunction (9)

|        | H   | H/M  | AI  | AR  | AI/AR | AI+J          | AR+J          | AI/H | AI/M | AR/H | AR/M |
|--------|-----|------|-----|-----|-------|---------------|---------------|------|------|------|------|
| Normal | 6.5 | 34%  | 3.5 | 5   | 70%   | 5.7<br>(+62%) | 6.2<br>(+24%) | 53%  | 18%  | 76%  | 26%  |
| Hyper  | 8.6 | 54.6 | 5   | 6.2 | 80%   | 6.5<br>(+30%) | 7.6<br>(+22%) | 58%  | 31%  | 71%  | 39%  |
| Hypo   | 6.6 | 47.3 | 1.5 | 3.3 | 45%   | 4.8<br>(+86%) | 4.9<br>(+48%) | 22%  | 10%  | 50%  | 23%  |

lumbo-sacral motoneurons strongly pre dominant for  $\gamma$ -static motoneurons and less pronounced for  $\gamma$ -dynamic and  $\alpha$ -motoneurons (Fig. 6)

On the other hand vestibular hypofunction following interruption of central vestibular pathways vestibular neurectomy or sectioning of the LVST at the spinal level becomes clinically manifest by the loss of postural control on the lesion side. Careful analysis in cases of minimal involvement shows that the slowness of the leg is due to hypotonia of extensor antigravitatory muscles accompanied by the loss of postural reflexes i.e. of the tonic component of the stretch reflex. This

constitutes what we call an abnormal Romberg test of negative type. Study of the phasic stretch reflexes shows a dramatic fall in excitability of  $\gamma$ -static motoneurons that can persist for months or even years. A less pronounced reversible fall in excitability of  $\gamma$ -dynamic and  $\alpha$ -motoneurons is also noticed (Fig. 7). Furthermore in cases of vestibular neurectomy when the fall in the value of AI is moderate or absent, the possibility of an incomplete neurectomy must be considered.

We are now performing a systematic study of stretch reflexes in every case of vestibular dysfunction where diagnosis is not possible and in every case in which surgery of the labyrinth

#### VESTIBULAR HYPERFUNCTION

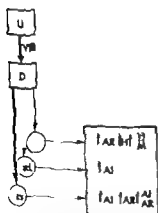


Fig. 6 In cases of vestibular hyperfunction the impulses from the utricle (U) and the lateral vestibular nucleus of Dorsal (D) provide state of hyperexcitability of alpha motoneurons (AI), gamma dynamic (AI) and gamma static (AR) motoneurons. The values of AI, AR and H are in these circumstances increased in relation to normal.

#### VESTIBULAR HYPOFUNCTION

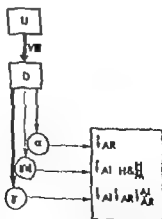


Fig. 7 In cases of vestibular hypofunction the excitability of  $\alpha$  and gamma motoneurons is decreased. The values of AI, AR and H are in these cases reduced in relation to normal.

ninth or vestibular nerve is considered Table V shows the preliminary result of this study series. In 13 cases in which the dominant symptom is vertigo the pattern of stretch reflexes corresponds to that of vestibular hyperfunction. In 9 cases of vestibular dysfunction in which chronic disequilibrium with unilateral positive Romberg test of deficitary type is present the pattern of myotatic reflexes corresponds to vestibular hypofunction. On the other hand there is no correlation between the electronystagmography and the stretch reflexes. Indeed a hyporeflexive labyrinth can be found in cases of vertigo and/or disequilibrium in which the pattern of reflexes is of hyperfunctional type.

A certain tendency towards a pattern of hyperexcitability of  $\alpha$ -motoneurons can be found in the hyperfunctional type according to the recovery curve of the H reflex (Fig. 5) whereas a strictly normal curve is found in the hypofunctional type.

## ZUSAMMENFASSUNG

Der Fasciculus vestibulo-spinalis lateralis (FVSL) scheint beim Menschen durch seine Erregung aus dem Utriculus in Antwort auf Verlagerungen des Gravitätszentrums eine Erhöhung des Erregungsniveaus der statischen  $\gamma$ -Motoneurone der Streckmuskulatur des Rumpfes und der unteren Extremitäten sowie wenn auch weniger ausgeprägt der dynamischen  $\gamma$  und der  $\alpha$ -Motoneurone zu erzeugen. Dies trägt dazu bei den Muskeltonus zur Beibehaltung der statischen Aufrechterhaltung und zum alternierenden Wechsel zwischen der gestreckten und gebeugten Phase während der Fortbewegung zu regulieren.

Eine übergehende vestibuläre Überfunktion bedingt durch zentrale oder periphere Stimulation äußert sich klinisch durch Schwindel. Während dieses Zustandes beobachtet man eine Übererregbarkeit der statischen  $\gamma$ -Motoneurone während die dynamischen  $\gamma$  und die  $\alpha$ -Motoneurone wenig oder gar nicht verändert sind.

Eine Aufhebung der vestibulären Funktion durch Zerstörung des Labyrinths, Durchtrennung des Nervus vestibularis, Unterbrechung der zentralen vestibulären Verbindungen oder des FVSL hat eine Gleichgewichtsstörung mit Verlust der Lagerungskontrolle zur Folge. In diesem Fall wird eine erhebliche Abnahme der statischen  $\gamma$ -Innervation registriert.

Das Vorhandensein einer adäquaten statischen  $\gamma$ -Innervation auf einer Seite scheint mit einer vollständigen Aufhebung der Labyrinthfunktion auf der gleichen Seite im Widerspruch zu stehen. Andererseits schließt das

Fehlen einer Antwort auf kalorischem Reiz in einem oder beiden Labyrinth die Möglichkeit einer vestibulären Überfunktion nicht aus.

Die Beobachtung der phasischen Muskeldehnungsreflexe scheint eine angemessene Methode zur Beurteilung des Funktionszustandes des statischen Labyrinths und des FVSL zu sein.

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## THE MECHANISM OF PHYSIOLOGICAL HEIGHT VERTIGO

## II Posturography

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(Received April 23 1979)

**Abstract** In order to validate the hypothesis that height vertigo is based on visual destabilization of free stance when the distance between eye and object becomes critically large several of its consequences were demonstrated in posturographic experiments. (1) Visual signals conflicting with simultaneous vestibular and somatosensory inputs provided by sinusoidally tilting rooms may destabilize postural sway in the fore-aft as well as in the lateral direction. (2) In natural surroundings sway amplitudes increase with increasing eye-object distance up to 5 meters. Thus teleologically subjective height vertigo serves as an appropriate warning signal to withdraw the body from a stimulus situation inducing postural imbalance. (3) Postural height vertigo problems can be alleviated (a) by adjusting the head relative to the gravitational vector and (b) by the presence of nearby stationary contrasts in the visual periphery according to the dominance of retinal periphery for dynamic spatial orientation.

The hypothesis in paper I Theoretical Approach and Psychophysics (Brandt et al 1979) provided the provocative prediction that at free upright stance the body sway will increase with increasing distance between the eyes and the nearest stationary objects within the visual scene. The present paper provides posturographic evidence supporting this hypothesis.

It has been argued that physiological height vertigo might be due to a specific insufficiency of the visual system: the limited resolution  $\Delta$  of the retina. Head movements cause a retinal slip of stationary visual targets. The retinal shift  $\alpha$  of a viewed stationary object in the environment depends on the lateral head displacement  $Y$  and on the eye-object distance

$X$ . A head or eye displacement  $Y$  is not perceived for  $\alpha < \Delta$ . In this latter situation there is no visual cue that could be used for postural compensation of this movement. The lack of a visual cue should result in larger disinhibited sway amplitudes at greater eye-object distances.

A series of experiments was designed to test whether height vertigo is associated with a visual destabilization of postural balance.

## METHODS AND RESULTS

*Stabilometry*

The fore-aft and lateral body sway were separately measured by use of a stabilometer (Kapteyn & de Wit 1972) at free upright stance. Stabilograms were both recorded by a strip chart recorder (Siemens EM 81 80) and stored on a magnetic tape recorder (Philips Analog 7) for further data analysis. Apart from the high frequency components the stabilograms very closely represent the movements of the centre of gravity and of the head as verified by means of a TV detection system when compared with the simultaneous stabilogram (Bles & de Wit 1976 Kapteyn 1973).

In order to enhance disproportionately the particular sensory weight of the visual information for postural control some of the experiments were performed with one or two pieces of foam<sup>1</sup> (height 10 cm, spec. wt

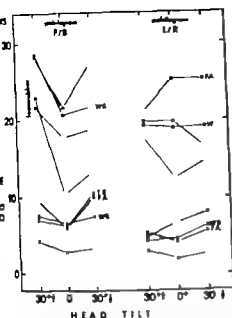


Fig. 1 The Root Mean Square (RMS) of lateral and fore-aft stabilograms obtained with upward or downward inclination of the head 30° out of the normal position (normal head position: ~15° tilted forward). The 4 subjects are standing with eyes closed both without ( ) and with (O) foamrubber on top of the stabilometer

40 g/dm<sup>2</sup>) placed on top of the stabilometer covered by a rigid plate. This makes the exteroceptive information of the ankle joints even less effective in maintaining postural balance.

#### Tilting rooms

In the laboratory the effects of misleading moving visual signals on posture were investigated by use of stabilometry during artificial movements of the viewed environment.

**Tilting Room I** is a motor-driven device (2.5 × 1.5 × 2 m) tilting laterally around an axis at ankle height with the subject standing on the stabilometer in front. The entire visual field covered by the stimulus device (Bles & de Wit, 1976). The room was tilted sinusoidally at a frequency of 1/45 Hz and amplitudes of either 1, 3.5, 5 or 10°.

**Tilting Room II** (Tönnies, Freiburg i.Br.) is completely closed except for a hole in the centre of the floor where the stabilometer is placed. This allows for lateral as well as fore-

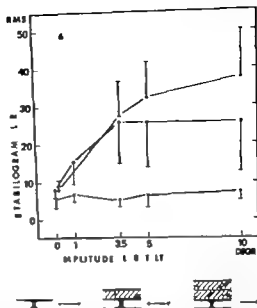


Fig. 2 RMS values (arbitrary units  $\pm 1\sigma$ ) of the lateral stabilogram obtained under sinusoidal lateral movement of Tilting Room I (freq 1/45 Hz, ampl 1, 3.5, 5 and 10°). The 8 subjects were standing both without (x), with single (O) and with double ( $\Delta$ ) foamrubber on top of the stabilometer.

aft optokinetic stimulation (Bles 1979). Stimulus frequency was 1/40 Hz with amplitudes of either 2, 4, 8 or 12°.

The subjects were requested to stand upright keeping optimal balance for at least three stimulation periods.

#### Computer-analysis of the stabilograms

The analysis of the stabilograms was performed using a PDP-8I computer system.

Fourier analysis of postural sway is very common nowadays (Diegenans et al. 1976; Leroux et al. 1973). The stationarity of the stabilogram which is required however is unlikely to be achieved during a one-minute registration period. Therefore we preferred a computerized analysis of sway amplitudes using Root Mean Squares (RMS) as a measure of stability (Kaptein & Bles 1976).

According to the hypothesis developed in paper I of this study we would expect to find a low frequency high amplitude body sway in

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In order to enhance disproportionately the particular sensory weight of the visual information for postural control some of the experiments were performed with one or two pieces of foamrubber (height 10 cm spec. wt

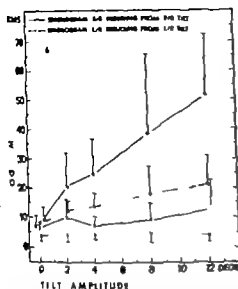


Fig. 4. Mean RMS values (arbitrary units,  $\pm 1\sigma$ ) of lateral and fore-aft stabilograms. Both the lateral and the fore-aft stabilograms are obtained from lateral resp. fore-aft sinusoidal tilt of Tilting Room II (freq. 1/40 Hz, ampl. 2, 4, 8 and 12°). The 6 subjects were standing both without (—) and with (---) foamrubber on top of the stabilometer.

attached to the head and restricting the visual field to 75°. For each condition six stabilograms were recorded, no one or two layers of foamrubber with or without a funnel. It must be emphasized that under these natural conditions it was not possible to control all stimulus parameters of the visual scene or to avoid occasional presence of a slight breeze.

As can be seen in Fig. 3 the instability is already maximal at an eye-object distance of 25 m. No significant difference between the RMS values for the different test conditions obtained at 25, 75 and 200 m could be established. This is also the case when peripheral vision is present. However, when using foamrubber the levels of instability (RMS values) obtained with peripheral vision are below the levels found with the restricted visual field ( $p < 0.01$ ).

The RMS of the stabilograms obtained in the eyes-closed conditions is of the same order of magnitude as the RMS obtained with eye-object distances of at least 25 m without peripheral vision. Ranking the subjects ac-

cording to the RMS in both the balcony and tilting room experiments gives a coefficient of rank correlation of 0.82, indicating that the role of vision in postural stabilization is subject-dependent. Since the postural instability is already maximal at a distance of 25 m the data of experiment I do not sufficiently determine the saturating relationship between eye-object distance and sway amplitudes below 25 m. Therefore a second experiment was designed for testing the influence of small distances as well as the differential effects of optokinetic stimulation on fore-aft and lateral body sway.

#### Experiment II. Lateral and fore-aft body sway at various distances (0.5 to 25 m) and proprioceptive interference

Six healthy subjects (4 male, 2 female, age 20–47) participated in this experiment.

First they were tested in Tilting Room II both with and without foamrubber. The results are shown in Fig. 4. There is a remarkable discrepancy with the results of Expt I. Although the RMS obtained with one layer of foamrubber in Tilting Room II is less than that found in Expt I, the subjects could not perform the test on double foamrubber. Most of the subjects preferred to have some assistance available even with only one layer of foamrubber because they felt unstable and sometimes experienced vertigo.

It is interesting to note that the subjects were much more sensitive to the fore-aft than to the lateral tilt.

The Tilting Room II experiment was followed by a more natural stimulus situation for testing the influence of the distance below 25 m under static conditions. In this experiment a university lecture hall was used to provide different eye-object distances thus eliminating the possibility of wind influencing the results. By a suitable choice of illumination and the use of an adapted funnel the eye-object distance could be varied up to 25 m. The distances in the lecture hall at which stabilograms were recorded were 0.5, 1.5, 5,



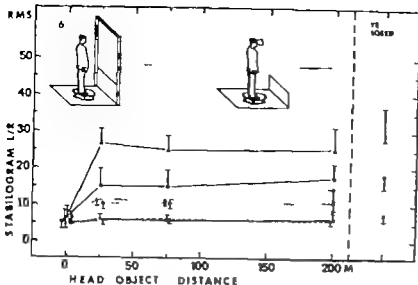


Fig 3 Mean RMS values (arbitrary units,  $\pm 1\sigma$ ) of lateral stabilograms obtained at different eye-object distances (0.5 25 75 and 200 m) without (—) and with ( ) the presence of nearby stationary contours in the periphery of the visual field. The 'eyes-closed' condition was tested as well. The 6 subjects were tested without (x), with one (O) and with two (Δ) layers of foamrubber on top of the stabilometer

conditions with considerable eye-object distance. This makes the choice of the RMS as a measure of stability in the following experiments acceptable. Since the RMS values represent an average over a certain period, they cannot be used for calculation of the net head movements (Y). But an increase in RMS values of the body sway is associated with an increase in head movements. The RMS values were computed from the last 45 sec of the one minute recordings in experiment I and from the last 40 sec in experiment II. In the tilting room experiments the RMS was computed for the second stimulus sinusoid in order to avoid possible transients.

#### *Pilot Experiment: Effects of head position on postural stabilization*

Frequently the head is bent or extended in height vertigo inducing stimulus conditions when looking up or down high structures. The net influence of the head position relative to the gravitational vector on posture was measured with eyes closed because with eyes open effects will be contaminated by additional changes in visual cues.

As can be seen in Fig 1 upward or downward inclination of the head by approx.  $30^\circ$  out of the normal position affects body sway—at least in the fore-aft direction. Stabilization is best with the otoliths in their normal work-

ing position (normal head position tilted forward  $\sim 15^\circ$ ).

#### *Experiment I: Lateral body sway at various eye-object distances (0.5 to 200 m) influence of peripheral vision and proprioceptive interference*

Six healthy students (4 male, 2 female, age 21–24) participated in this experiment. They first were tested in Tilting Room I while standing both with and without foamrubber to check on the proprioceptive interference during visual destabilization.

The results in Fig 2 show that left/right stability of the stabilogram without foamrubber does not increase very much despite the increasing amplitude of the room tilt. With foamrubber a maximum level of RMS values indicating proprioceptive interference is reached already at an amplitude of  $3.5^\circ$  of room tilt. With double foamrubber this occurs at a higher level as was to be expected. The considerable standard deviations suggest large interindividual differences.

Subsequently by placing the stabilometer on balconies of a high building it was possible to vary the distance between the eye and the visible surroundings from 0.5 25 75 up to 200 m. Nearby stationary visual cues from the retinal periphery (balustrade and ceilings of the balcony) could be occluded by a funnel

closure during pathological vestibular vertigo. This explanation in fact fits earlier results in patients with postconcussional dizziness or peripheral lesions when exposed to artificial around motion in Tilting Room I (Bles 1977, Bles et al. 1977). These patients probably because of the lesional dysfunction of the vestibular system exhibited similar symptoms even without use of foamrubber.

The lower saturation levels of RMS values (see Expt I) as obtained in the peripheral vision-foamrubber conditions are visually determined and therefore can be interpreted as levels of visual interference. This is true because on excluding peripheral vision the stability increases significantly up to the level of proprioceptive interference. Without foamrubber no difference in stability could be established indicating that the level of proprioceptive interference here is lower than that of peripheral vision. In the condition without foamrubber and with peripheral vision there is no chance that a perceptual conflict arises because with the nearby stationary contrasts in the visible periphery body movement is detected by both proprioception and vision.

The posturographic data support the hypothesis that physiological height vertigo is associated with a postural imbalance. It was shown that lateral and fore-aft body sway as well as head sway increase non-linearly with increasing eye-object distance and that nearby stationary contrasts in the periphery of the visual field reduce the body sway.

The static stimulus condition of height or distance will not *per se* destabilize posture to an irresistible fall because of the redundancy in the control system. The lack of an appropriate input is widely compensated for by the somatoreceptors and the labyrinths. However additional disturbances like wind or an unstable food support may result in serious problems in maintaining the upright position as subjects are apt to use the 'false visual information in problematic circumstances. Therefore patients with vestibular or somatosensory dysfunctions (e.g. polyneuropathy)

are subjected to a particularly greater risk when exposed to height vertigo situations. This also explains the results of Pogany (1958) who described different sensory deficits among his population of sufferers from height vertigo. Because the postural behaviour of the subjects in the tilting room experiments and in the experiments with the varying eye-object distances was similar (large inter but small intra-individual differences) it is expected that subjects experiencing difficulties in the tilting room will also have difficulty in stabilizing posture when the critical eye-object distance is exceeded. Thus the tilting room could serve to predict the individual susceptibility to physiological height vertigo. The finding that under static conditions with eye-object distances exceeding 5 m vision does not seem to play any part in postural stabilization suggests that then larger retinal shifts ( $\alpha$ ) are required as hypothesized in paper I.

The posturographic data of the present study subserve the conclusions reached in paper I which were based on psychophysical data. Height vertigo can be minimized by

1. adjustment of the head to the gravitational vector when looking down since head tilt reduces postural stability by bringing the otoliths out of their optimal working range and therefore disproportionally enhancing the visual contribution to postural stabilization (pilot experiment)
2. providing nearby stationary cues in the periphery of the visual field since they provide peripheral vision with the necessary information to stabilize posture (Expt I)
3. avoiding free stance under height vertigo conditions because of the destabilizing role of vision under such circumstances (Expts I and II)

The physiological vertigo is primarily a result of the induced imbalance. If the subject is lying down incongruity of the proprioceptive and visual information does not exist. The occurrence of physiological height vertigo is then unlikely. This conclusion based on pos-

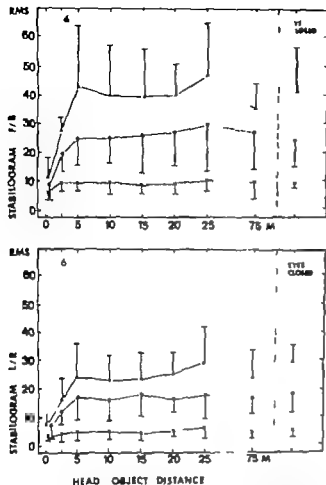


FIG 5. Mean RMS values (arbitrary units,  $\pm 1\sigma$ ) of lateral and fore-aft stabilograms obtained at different eye-object distances (0.5, 5, 10, 15, 20, 25 and 75 m) both without (O) with single (O) and with double (Δ) foamrubber on top of the stabilometer. The 6 subjects had a restricted visual field of 75° by means of a funnel attached to the head. The eyes-closed condition was tested as well.

10, 15, 20 and 25 m. The eyes-closed condition was tested as well. These measurements were performed with the stabilometer with out with single and with double layers of foamrubber. For comparison these subjects were also tested at the 75 m condition of Expt I.

As can be seen in Fig. 5 the instability in the fore-aft direction is greater than in the left-right direction. The results confirm the findings of Expt I in that the maximum RMS at an eye-object distance of at least 5 m are of the same order as the RMS obtained at the 75 m condition on the balcony. Only the point of saturation shifted to the smaller distance of 5 m.

## DISCUSSION

Active movement of the seen environment may induce an apparent self motion sensation in the opposite direction followed by a postural reaction with a tendency to fall into the direction of pattern motion. Such destabilizing visual effects have been demonstrated in several experiments (Dichgans et al 1976, Lee & Lishman 1975, Lestienne et al 1976). The tilting room however is the most natural stimulation since motion is about an axis at ankle height (Bles 1979, Bles et al 1977). Increasing tilt angles result in increasing visual-proprioceptive conflicts which permit an evaluation of multisensory interference on postural stability. The results of Expt I with Tilting Room I reveal a level of proprioceptive interference which is relatively independent of the increasing amplitude of the sinusoidal tilt. No vertigo was experienced by the subjects, indicating that these stimulus conditions were not particularly stressful. It must also be emphasized that despite the fact that the subjects knew about the tilting of the room, the body sway was influenced by the stimulus especially when standing on foamrubber. This is consistent with the earlier findings obtained with Tilting Room I (Bles & de Wit 1976).

Expt II using Tilting Room II did not reveal a clear level of proprioceptive interference in the foamrubber conditions. However in this experiment some of the subjects reported vertigo up to a complete loss of spatial orientation accompanied by distressing symptoms of optokinetic motion sickness.

We believe that the foamrubber platform reducing the reliability of the joint receptors consequently reduces the redundancy of the reafferent sensory signals which are used for multisensory control of posture. Then vertigo and postural destabilization are mainly due to the increased sensorial weight of the misleading visual signal which is contradicted only by the correct vestibular cues. Thus the intensity of disequilibrium is a function of the magnitude of the mismatch and is increased if one of the sensory systems is eliminated such as with

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otic hydrops can occur in both congenital and acquired manifestations may lie in the etiology. Eight endolymphatic decompression procedures were performed on 10 patients with refractory luetic vertigo. Of these, 3 had congenital and 3 acquired lesions. All cases had received adequate medical therapy without any improvement in the vertiginous attacks. In our 6 cases of congenital hydrops the round window was closed and the round window membrane was removed. In our 3 cases of acquired hydrops the round window membrane was removed and the round window was closed. In all cases vertigo was controlled or eliminated in all cases. Hearing was improved in one patient, but not in the other two. Generally speaking, we recommend endolymphatic decompression as a procedure of last resort for patients with refractory luetic vertigo who have not responded to adequate medical therapy.

otic hydrops may mimic the symptoms of Meniere's disease. However, luetic hydrops is characterized by bilaterality, earlier onset, and progressive symptoms. The most distinguishing symptom of luetic hydrops is episodic vertigo.

Endolymphatic sac decompression procedures were done on 6 patients with refractory luetic vertigo in an attempt to relieve recurrent vertiginous attacks which were refractory and unresponsive to conservative medical therapy. We also hope to prevent further hearing impairment.

In this report we intend here to describe this procedure and to recommend consideration of endolymphatic decompression of the endolymphatic sac in patients with luetic vertigo where extensive medical management has hitherto failed.

## REVIEW OF THE LITERATURE

Luetic hydrops may be seen in both congenital and acquired syphilis. In 1966, Karmody & Schuknecht reported that 38% of 123 congenital syphilitics had a hearing loss, most of whom had associated vestibular symptoms with episodic vertigo and diminished caloric responses. They demonstrated a case of endolymphatic hydrops due to syphilitic osteitis of the otic capsule. Similar findings were observed by Alexander (1928) and Mayer & Fraser (1936). Perlman & Leek (1957) stated that endolymphatic hydrops secondary to invasion of the labyrinthine capsule may have some resemblance to the changes reported in Meniere's disease. This may account for the occasional similarity of symptoms. Schuknecht (1974) suggested that the inner ear reaction to syphilis is characterized by progressive endolymphatic hydrops and degeneration of the membranous labyrinth, resulting in rupture of the membranes due to overaccumulation of endolymph.

Pulec (1977) reported that 7% of his patients with Meniere's syndrome complex were found to have a syphilitic etiology and Shea & Bowles (1975) showed that 12.5% of the patients with a fluctuating hearing loss had a syphilitic origin. Severe episodic vertigo with nausea and vomiting in congenital syphilis is indistinguishable from Meniere's disease and it may precede hearing loss by months or years and

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## ZUSAMMENFASSUNG

Die Ergebnisse posturographischer Untersuchungen stimmen mit der Hypothese überein, daß dem physiologischen Hörschwindel eine visuelle Destabilisation des aufrechten Standes zugrunde liegt, wenn der Abstand zwischen Auge und stationären Umweltkontrasten kritisch groß wird. 1) Strömungsartige Kippbewegungen eines künstlichen Raumes führen zu einem intersensorischen Wahrnehmungskonflikt zwischen visuellen vestibulären und somatosensorischen Meldungen mit optokinetischer Haltungsdestabilisierung in vorwärts-rückwärts oder seitlicher Richtung. 2) In einer natürlichen Umgebung nehmen die Schwankamplituden mit zunehmenden Auge-Objekt-Abstand (bis zu 5 m) zu, d.h. unter hörschwindelauslösenden Reizbedingungen besteht eine reale Fallgefahr. 3) Die Haltungsregulation kann verbessert werden: a) durch eine natürliche aufrechte Kopfhaltung gegenüber dem Gravitationsvektor, b) durch nahe stationäre Kontraste im peripheren Gesichtsfeld entsprechend der Dominanz der peripheren Retina für die dynamische Raumorientierung.

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Luetic hydrops can occur in both congenital and acquired forms and otologic manifestations may be indistinguishable from Meniere's disease. Eight endolymphatic decompression procedures were performed on 5 with refractory luetic vertigo. Of these, 3 had acquired and 3 acquired luetic. All cases had received extensive medical therapy without any improvement in the vertiginous attacks. In our 6 with luetic hydrops the main complaint was episodic vertigo, indeed disabling in most cases. In all cases remained free from these severe and the vertigo was controlled or eliminated in all. Although hearing was improved in one patient, seemed not to improve generally speaking. As if the long-term postoperative follow-up. We do not recommend of sac decompression and drainage and that this procedure might be considered for patients with uncontrollable luetic vertigo who have not responded to adequate medical therapy.

Luetic hydrops may mimic the symptoms of Meniere's disease. However, luetic hydrops is characterized by bilaterality, earlier onset, and slowly progressing symptoms. The most distinguishing symptom of luetic hydrops is episodic attacks of vertigo.

Eight endolymphatic sac decompression procedures were done on 6 patients with refractory luetic vertigo in an attempt to relieve recurrent vertiginous attacks which were disabling and unresponsive to conservative treatment. We also hope to prevent further hearing impairment.

It is our intention here to describe this procedure and to recommend consideration of surgical decompression of the endolymphatic sac in patients with luetic vertigo where extensive medical management has hitherto failed.

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Endolymphatic sac decompression procedures were done on 6 patients with refractory luetic vertigo in an attempt to relieve current vertiginous attacks which were disabling and unresponsive to conservative treatment. We also hope to prevent further hearing impairment.

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may occur without hearing impairment. In children with congenital syphilis the onset of deafness is not accompanied by marked vestibular symptoms such as is found in the adult group (Karmody & Schuknecht 1966).

The Tullio sign occurs more often in congenital syphilis (Schuknecht 1974). A positive fistula test without fistula or Hennebert's sign has been considered to be pathognomonic of congenital syphilis (Karmody & Schuknecht 1966; Perlman & Leek 1952). Nadol (1974) described fibrous adhesions between the footplate and membranous labyrinth and assumed this to be the pathological basis for Hennebert's sign. This finding was common in congenital syphilis, viral labyrinthitis and Meniere's disease. Fiumara & Lessell (1970) analysed 271 patients with late congenital syphilis and 9 (3%) were found to have a hearing loss. In these cases the onset was ushered by vertigo followed by hearing impairment.

Hearing loss is reported to occur in about 25% to 38% of patients with congenital syphilis (Karmody & Schuknecht 1966). Tamari & Itkin (1951) reported the incidence of hearing loss in syphilis as 18% in late congenital, 17% in early latent, 25% in late latent, 29% in asymptomatic syphilis and 80% in symptomatic syphilis.

Patterson (1968) summarized the key to diagnosis of congenital luetic hearing loss as a high index of suspicion, a sudden bilateral symmetrical sensorineural hearing loss with or without vertigo in childhood, a sudden gradual asymmetrical sensorineural hearing loss with wide fluctuation and poor discrimination with or without vertigo in adult and positive serologic tests.

Patients with luetic hydrops have traditionally been treated with extensive and if necessary prolonged medical therapy. However, in 1975 Shambaugh reported one patient who had congenital syphilis with labyrinthine hydrops who underwent an endolymphatic sac decompression procedure in his 105 cases of sac decompressions.

In 1976 the senior author reported on 3

cases of luetic hydrops among his series of endolymphatic sac decompressions.

We could find no previous specific report in the literature of surgical decompression for cases of refractory luetic endolymphatic hydrops.

## CLINICAL REPORTS

The senior author has performed endolymphatic sac decompression procedures in 11 cases of endolymphatic hydrops. Eight ears of 6 patients with refractory vertigo due to luetic endolymphatic hydrops (5 female and 1 male) are herein described.

The method of decompression utilized has been described previously and represents modification of the methods of Portmann and House (1962). Three basic features of the technique are as follows.

- 1 Wide exposure of the dura around the endolymphatic sac which means all of Trautmann's triangle including the infralabyrinthine area and the adjacent margin of the lateral sinus.

- 2 Avoiding skeletonization of the posterior semicircular canal. Approaching the sac from behind, leaving the canal imbedded in the solid bone of contiguous facial buttress.

- 3 Draining the sac with a T-tube into the mastoid cavity fashioned out of Silastic sheeting which is held in place by the dura and overhanging bony edge.

Three patients (cases 1, 2, 3) aged 56, 34 and 55 had late congenital lues with the first sign of interstitial keratitis at age 6, 18 and 36. Hutchinson's teeth were found in case 2. The remaining cases (4, 5, 6) aged 55, 58 and 56 (male) had acquired lues. Serologic tests were positive in blood and negative in CSF in all cases (Table I).

All cases had an audiovestibular symptom complex typical of idiopathic endolymphatic hydrops (Meniere's disease). The most disabling complaint at admission was long-standing incapacitating vertigo in all cases. The vertigo was most often sudden.

Table 1 Clinical findings in 6 patients with refractory luetic vertigo

| No.   | Age & Sex | Race | First visit | Diagnosis & Findings  | Blood |         | CSF  |                          |
|-------|-----------|------|-------------|---|-------|---------|------|--------------------------|
|       |           |      |             |   | VDRL  | FTA ABS | VDRL | VFT                      |
| 1 A V | 36 F      | W    | Apr 1976    | Congenital luetic late interstitial keratitis at 6 years  | -     | 2+      | -    | Bilateral hyporeactivity |
| 1 J G | 34 F      | W    | Apr 1970    | Congenital luetic late interstitial keratitis at 18 years. Hutchinsonian teeth                    | +     |         | -    | Bilateral hyporeactivity |
| 1 G S | 55 F      | W    | Nov 1968    | Congenital luetic late interstitial keratitis at 36 years. Cerebellar degeneration. Fister sign + |       | 1+      | -    | Bilateral hyporeactivity |
| 1 E   | 55 F      | W    | Aug 1973    | Acquired luetic late latent at 19 years   | +     | 4       |      | Bilaterally reduced      |
| 1 L   | 58 F      | W    | Jan 1973    | Acquired luetic late serous otitis  | ++    | 3       |      | Bilateral hyporeactivity |
| 1 K   | 46 M      | W    | Jan 1971    | Acquired luetic late latent   |       | 4+      | -    | Bilateral hyporeactivity |

impaired by nausea and vomiting. Dyplasia was demonstrated in cases 5 and 6. Headache and pressure were present in 3 cases. Hearing loss was first noticed in one ear (aged 38, 36 and 50 in congenital luetic; 50, 54 and 43 in acquired luetic) with tinnitus and pressure sensation and then in the other ear with progressive deterioration of hearing. Audiometry revealed sensorineural loss in all cases with high tone loss at first examination in cases 1, 3 and 6 and flat loss in others. Hearing thresholds fluctuated and progressively deteriorated along with discrimination scores (14% in case 3 and 16% in case 5). SISI scores were very high in all cases including the unoperated ears and Békésy tests showed Type II in both ears of case 5. Hearing loss was severe in one ear at first examination in cases 1, 3 and 6. All cases received extensive anti-luetic treatment. Case 1 showed improved hearing with an increased SRT (45 dB to 15 dB) and discrimination score (97% to 100%) in her left ear following an 8 day course of penicillin and ACTH therapy. However progressive deterioration of SRT (45 dB to 70 dB)

and the discrimination score (88% to 50%) were noticed in the left ear without any improvement in the character or frequency of vertiginous episodes.

The postoperative period of follow-up varies between 3 and 8 years. In some patients a sac decompression procedure in one ear (the worse ear) appeared to control the vertigo and the opposite side therefore did not receive surgery. Although hearing was improved in one patient (25% increase in discrimination) it was preserved in all cases. In general progressive hearing loss developed over the years subsequent to the procedures.

The following two representative patients are described in greater detail.

#### Case 1

S A V white female age 56 a known congenital luetic with bilateral interstitial keratitis at age 6 had an onset of episodic whirling vertigo, nausea and vomiting at age 16, occurring every few months lasting for a few hours. A bilateral progressive hearing loss was first noticed 18 years ago in the left ear accompanied by continuous tinnitus. Hearing

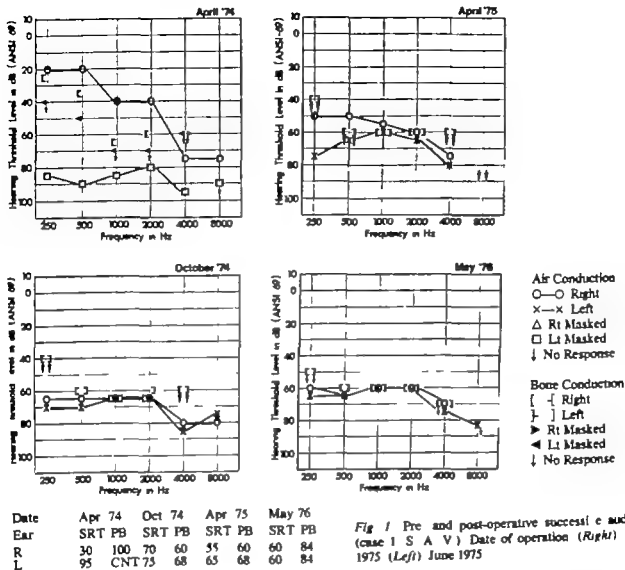


Fig 1 Pre and post-operative successive audiograms (case 1 S A V). Date of operation (Right) October 1975 (Left) June 1975

had recently worsened in her right ear which was her better ear (Fig 1). VDRL was negative and the FTA ABS test was 2+ in blood and VDRL was negative in CSF in June of 1974. She received a 10 day course of Bicillin and Prednisone therapy in October 1974. Bithermal air caloric testing showed bilateral severely reduced vestibular function. An audiogram revealed a bilateral sensorineural hearing loss with an SRT of 55 dB (R) and 65 dB (L) and discrimination scores of 60% (R) and 68% (L).

She was admitted with a diagnosis of congenital lentic hydrops with refractory vertigo and a 10-day course of Potassium Penicillin G and Solu-cortef was administered parenterally. Her hearing thresholds remained unchanged on serial audiometric evaluation.

Bilateral endolymphatic sac decompression was performed in June (L) and October 1975. Postoperatively she had a mild dizziness feeling after her first operation but since second operation she has remained free from vertigo and her hearing was stabilized with SRT of 60 dB with elevated discrimination scores (84% on both ears) at her most recent follow-up.

This next patient was extremely incapacitated her vertigo was aggravated by riding in a car. She had to be practically carried to the clinic for initial visits.

#### Case 4

V E white female age 55 had an onset of periodic vertigo with accompanying nausea and vomiting and fullness in her ears. Last

up to 3 hours at age 25. Her hearing began to deteriorate progressively in both ears starting 5 years ago. It was fluctuating and progressive, worse on the right ear after a flu-like illness. She had syphilis at age 19 and was treated with Bismuth therapy. She had also received a 70-day course of penicillin therapy 10 years ago.

On admission (August, 1973) audiometric examination revealed a bilateral sensorineural hearing loss with an SRT of 60 dB (R) and 70 dB (L). Discrimination scores were 54% (R) and 58% (L) and SISI scores were 100% on both ears at 4 kHz. Caloric response was markedly reduced bilaterally with very slight subjective sensation in the left ear and none in the right ear. VDRI was reactive and the FTA-ABS test was 4+ in blood and VDRI was negative in CSF. Bacillin was given for 4 weeks with a diagnosis of late latent acquired syphilis. Subsequently additional medical therapy failed to control her symptoms and an endolymphatic sac decompression procedure was performed in September 1973 on the right ear and in February 1974 on the left ear.

She is now rehabilitated. Postoperatively she has had a mild sensation of pressure in her ear and slight imbalance. However she has had no further vertiginous attacks and she is very happy being able to drive her car again.

## DISCUSSION

Histopathologically the lesion of congenital syphilis cannot be differentiated from that of the acquired type. It is characterized by progressive hydrops of the endolymphatic system, degeneration and atrophy of labyrinthine structures with rupture of the membranous labyrinth, causing severe incapacitating episodic vertigo accompanied by nausea and vomiting, fluctuating hearing loss and reduced speech discrimination. Tinnitus, loudness intolerance, sensation of fullness in ears, and diplacusis are also present as in Meniere's

disease. Among those symptoms the most disabling symptom in luetic hydrops is refractory vertigo.

Of our 5 patients, 4 cases initially presented with vertiginous episodes and 2 with cochlear symptoms (hearing impairment and tinnitus). However at the time of admission the patients' chief complaint was incapacitating episodic vertigo which could not be controlled by conservative measures such as penicillin, steroids, erythromycin, antiemetics, sedatives or tranquilizers. Three patients were diagnosed as late congenital syphilis with interstitial keratitis and Hutchinson's teeth (case 2) and the other 3 patients as acquired syphilis.

Intensive specific anti-luetic medical therapy may fail to prevent the onset of luetic vertigo, deafness or to stabilize the disease process. A hearing loss may occur despite adequate previous antibiotic or steroid therapy due to the variable response of the disease. Our patients had received previous anti-luetic treatment without any significant effect in controlling incapacitating vertiginous episodes or progressing hearing impairment.

In inadequately treated syphilis, viable spirochetes are occasionally found in eye, CSF, synovial fluid and lymph nodes and in the temporal bones of penicillin-treated congenital syphilitic deafness. Spirochetes have been found in patients adequately treated with heavy metals. Tamari & Ilton (1951) reported that despite administration of 9 million units of penicillin in 15 days, both hearing and vestibular function continued to deteriorate. The effective level of penicillin in vitreous humor, spinal fluid or labyrinthine fluid against the spirochete is still open to question.

The patient who is allergic to penicillin has a serious problem in treatment. South et al (1964) reported a case of a gravid woman treated with 15 g of erythromycin estolate over a 10-day period 2 months prior to delivery. Her syphilitic infant died and the fetal level of erythromycin was only 1/5 to 1/20 of the maternal level. Case 5 was allergic to penicillin and she was treated with erythromycin for

15 days prior to scheduling endolymphatic sac decompression

We considered parenteral streptomycin therapy to ablate the vestibular labyrinth in these patients. This was done in one patient in this series and in 2 patients with intractable bilateral Meniere's disease without success. Thus in our opinion the sac decompression seemed to be the procedure of preference.

It is difficult to describe the physiological rationale of why a unilateral procedure would benefit the patient with bilateral disease. These procedures were all performed for refractory vertigo. This same observation was made in a study of certain patients with bilateral Meniere's disease. From this small series of cases we have not determined that the procedure is beneficial for improving hearing in patients with luetic hydrops. The procedure can be of unquestionable value to patients who have refractory vertigo however due to luetic hydrops where medical therapy has failed.

## ZUSAMMENFASSUNG

Lues bedingter Hydrops kann in der angeborenen und erworbenen Form auftreten und die otologischen Befunde sind oft sehr schwierig von der Menstrischen Krankheit zu unterscheiden. Acht endolymphatische Sack Decompressions-Operationen wurden an 6 Patienten mit refraktären luetischen Vestibularisstörungen ausgeführt. Drei Patienten hatten Lues congenitalis und drei die erworbene Form. Alle Patienten waren ausgiebig mit antiluetischen medizinischen Mitteln konservativ behandelt worden, ohne jedoch irgendwelche Besserung der Schwindelanfälle aufzuweisen. In unseren 6 Patienten mit luetischem Hydrops waren die Schwindelanfälle so intensiv und häufig, daß sie in fast allen Fällen zur Berufsunfähigkeit führten. Alle Patienten hatten postoperativ keine Vestibularisstörungen. Das Hörvermögen war in einem Patienten besser und in den anderen Patienten war keine Hörverbesserung im Long-term follow-up aufzuweisen. Unsere Operationsmethode zur endolymphatischen Sack Decompression und Drainage wurde veröffentlicht und kann in Patienten mit unkontrollierbarem luetischem Vertigo angeführt werden, nachdem die extensive medizinische Behandlung erfolglos war.

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## COMPUTER ANALYSIS OF HOARSENESS

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**Key words:** The harmonic components in hoarse voice were studied from the noise components by using a small story computer. The ratio of harmonics to noise (ratio) was calculated and compared with the auditory impression for 58 subjects. The calculated results showed definite correlation to the auditory impression. It is suggested that this would be a useful method for tentative evaluation of hoarseness.

Hoarseness is a symptom common to almost all types of laryngeal ailments. There have been many attempts to evaluate hoarseness as an aid to diagnosis, and these analyses have been significantly advanced by the introduction of the computerized spectrograph (Nessel, 1960; Ishiki et al., 1966; Hiroto, 1967; Yanagihara, 1967; Kato et al., 1970; Rontal et al., 1975).

A spectrogram of a normal sustained vowel, when seen with a narrow filter, shows well developed harmonics as equally spaced horizontal stripes, while that of a hoarse voice presents noise components as a cloudy shadow between the harmonics. Harmonic components are replaced by noise components as the degree of hoarseness becomes more severe (Fig. 1). If the acoustic energy of the harmonics is calculated separately from that of the noise components, the degree of hoarseness can be determined as a ratio of harmonics/noise or the signal/noise ratio.

This study was undertaken to devise a method by which to obtain the objective evaluation mentioned above through the use of a digital computer.

*Spectral Analysis*

A periodic waveform can be represented as a sum of sinusoidal waves, and each component

sinusoid is called a harmonic of the waveform. Fourier Series Expansion is a basis for this harmonic analysis. Its application, however, is restricted to a periodic waveform.

In speech analysis, Fourier Integral Formula or Fourier Transformation, which can be applied to an aperiodic waveform, is preferred. Since the acoustic speech waveform constantly changes its characteristics as the articulators shift position, it is practical to observe a short-term spectrum continuously.

One way to obtain a short-term spectrum is to isolate a certain time interval (time window) from the waveform, so that the waveform is zero everywhere outside the interval. There is a relationship of inverse proportion between a time interval ( $\Delta t$ ) and the bandwidth of the spectrum ( $\Delta \omega$ ) as follows:

$$\Delta t \Delta \omega = 1$$

From this equation it is concluded that a wide time window provides better frequency resolution, while a narrow time window produces better temporal resolution. Therefore, the time interval should be determined by the purpose of the investigation.

Since our aim in this study is to separate harmonics from noise components and calculate the signal/noise ratio, it is desirable to set a wide time window and to use a tone

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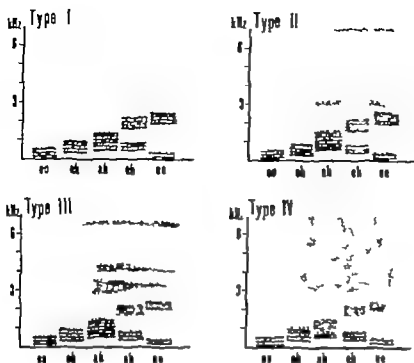


Fig 1 The four grades of hoarseness (from Yanagihara 1967)

which makes few articulatory changes during phonation such as a sustained vowel. Since even a sustained vowel changes its frequency composition gradually because of vibrato or trend, the interval of the time window has to be restricted, and from our experience it is assumed to be 20–30 msec.

Suppose that the voice waveform is analysed by Fourier Transformation utilizing a time window of 20–30 msec. In this manner a continuous spectrum is obtained in which the individual harmonic components have a frequency bandwidth determined by the time window, i.e. the narrower the time window, the wider the bandwidth. This spectrum is unusable for our study because the wide fre-

quency bandwidth makes it difficult to separate the harmonic energy from the noise (Fig 2).

Fourier Series Expansion is used in this study because it offers a dispersal spectrum with good frequency resolution. Since Fourier Series Expansion requires a periodic waveform, the following idea was designed.

Three pitch periods ( $T$ ) were extracted from the voice waveform and repeated endlessly. This results in the formation of a periodic wave which can then be subjected to analysis. The display shows well defined harmonics and the noise energy can be easily recognized and

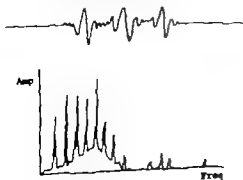


Fig 2 Waveform analysed by Fourier Transformation

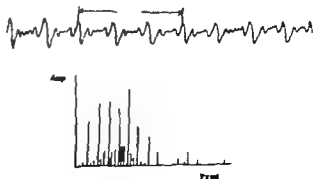


Fig 3 Periodic waveform analysed by Fourier Series Expansion.

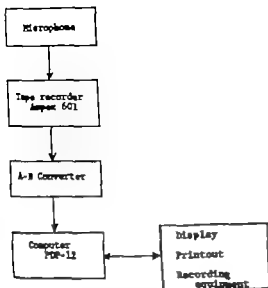


Fig 4 Block diagram of the system

extracted. It should be noted that the third harmonic on the display is actually the first harmonic of the voice waveform (Fig. 3).

## METHOD

A block diagram of the system is shown in Fig. 4.

### 1. Voice recording

The subjects were 14 males and 14 females with no laryngeal or pulmonary disorders and 20 males and 10 females with various laryngeal diseases resulting in various degrees of hoarseness (Table 1).

A recording of each subject's voice was

Table 1 Types of voice disorders

| Type of disorder          | N of cases |
|---------------------------|------------|
| Recurrent nerve paralysis | 11 (2)     |
| Atrophy                   | 7 (3)      |
| Polyp                     | 7 (1)      |
| Polypoid degeneration     | 1          |
| Carcinoma                 | 1          |
| Nodule                    | 1          |
| Acute laryngitis          | 1          |
| Matutinal voice disorder  | 1 (1)      |

Numbers in the parentheses represent postoperative patients.

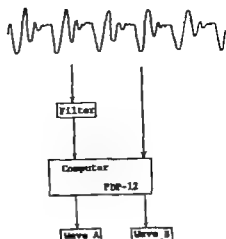


Fig 5 Voice wave digitized without filter (B) and with filter (A)

made in a sound-treated booth using a Electrovoice model 666 microphone and an Ampex model 601 tape recorder. A stable portion of the sustained vowel /a/ was used for the analysis.

The voices on the magnetic tape were edited and arranged randomly in order to avoid listener judgment bias. Five persons who had experience in voice study were asked to judge the degree of hoarseness for each subject. The degree of hoarseness was rated as follows: 0=none, 1=slight, 2=fair, 3=extreme, and the scores were averaged for each subject.

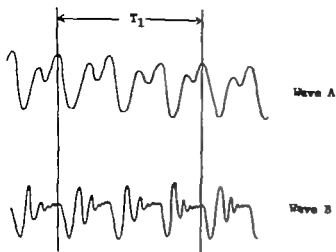
### 2. A/D conversion

A 325 msec section of the voice signal was filtered with a 600 Hz low pass filter and digitalized (wave A). The same section of the voice signal was digitalized without filtering (wave B) (Fig. 5). The sampling rate of the A/D conversion was 22026 points per second. This rate was determined by the sampling program and both waves were stored on computer data tape.

### 3. Pitch extraction

Wave A was displayed on a scope and three pitch periods ( $T$ ) were measured using a cursor. Fourier analysis was performed on the



Fig 6 The calculation of  $T_1$ 

corresponding pitch periods ( $T_1$ ) of wave B (Fig 6)

#### 4 Fourier analysis

If the three pitch periods ( $T_1$ ) of wave B are repeated endlessly the result can be considered a periodic wave  $f(t)$  whose pitch is  $T_1$  where frequency is  $F=1/T_1$ . Therefore this wave  $f(t)$  can be expanded into a series of sinusoidal waves whose pitch periods are  $T_1, T_1/2, T_1/3, \dots, T_1/n$  using Fourier Expansion as follows

$$f(t) = \frac{A_0}{2} + \sum_i \left( A_n \cos \frac{2n\pi}{T_1} t + B_n \sin \frac{2n\pi}{T_1} t \right) \\ = \frac{A_0}{2} + \sum_i X_n \sin \left( \frac{2n\pi}{T_1/n} t + E \right)$$

where

$$A_n = \frac{2}{T_1} \int_0^{T_1} f(t) \cos \frac{2n\pi}{T_1} t dt$$

$$B_n = \frac{2}{T_1} \int_0^{T_1} f(t) \sin \frac{2n\pi}{T_1} t dt$$

$$X_n = \sqrt{A_n^2 + B_n^2}$$

#### Calculation

Since the fundamental pitch of the actual voice wave is  $T_1/3$  (fundamental frequency  $\approx 3F$ ) harmonic components appear on the spectrum

of  $f(t)$  at  $3F, 6F, 9F, \dots$  and between these harmonics are noise components. In this study the wave  $f(t)$  was expanded into sinusoidal waves with harmonic frequencies up to 5500 Hz, and this determined the value of  $n$ . The signal/noise ratio of  $T_1$  was calculated as follows

$$R_1(\text{dB}) = 10 \log_{10} \frac{S_1}{N_1}$$

where

$$S_1 = X_3^2 + X_6^2 + X_9^2 + \dots$$

$$N_1 = (X_1^2 + X_2^2) + (X_4^2 + X_5^2) + (X_7^2 + X_8^2) + \dots$$

This procedure was repeated on the next three pitch periods ( $T_2$ ) and continued one after another ( $T_3, T_4, T_5, \dots, T_m$ ) for 325 msec. In order to obtain the average  $S/N$  ratio  $R$  was calculated as

$$R_n(\text{dB}) = 10 \log_{10} \frac{S_1 + S_2 + S_3 + \dots + S_m}{N_1 + N_2 + N_3 + \dots + N_m}$$

## RESULTS

The  $R$  value for 28 normals ranged from 15.0 to 23.5 dB, and Fig 7 shows the distribution. The critical region of normal was 15.4 to 23.3 dB at a 5% significance level, and no statistical difference was found between males and females.

Table II presents the  $R$  values of patients' voices along with the auditory impression for each voice. Six subjects show values within

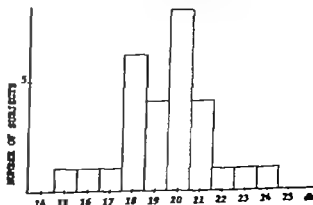


Fig 7 Histogram of normals.

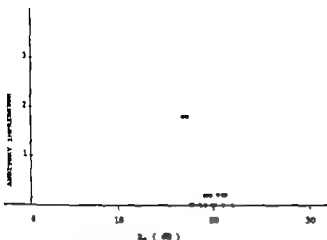


Fig. 8. Comparison of  $R_s$  value to listener judgment.

the critical region of normal. The overlap is not unexpected because in this study the pathologic group consisted of different stages of various disorders including postoperative voices. The breakdown of pathologies of the

Table II. The  $R_s$  value and auditory impression of pathologic voices

| Subject no | $R_s$ (db) | Auditory impression |
|------------|------------|---------------------|
| 1          | 12.6       | 2.0                 |
| 2          | 3.9        | 2.4                 |
| 3          | 12.5       | 2.2                 |
| 4          | 10.1       | 2.0                 |
| 5          | 4.7        | 2.8                 |
| 6          | 1.5        | 3.0                 |
| 7          | 16.8       | 0.8                 |
| 8          | 13.8       | 2.0                 |
| 9          | 1.7        | 2.0                 |
| 10         | 17.3       | 1.8                 |
| 11         | 17.0       | 1.8                 |
| 12         | 20.3       | 0.6                 |
| 13         | 3.5        | 1.4                 |
| 14         | 0.9        | 3.0                 |
| 15         | 11.3       | 2.4                 |
| 16         | 4.5        | 3.0                 |
| 17         | 13.9       | 2.4                 |
| 18         | 2.5        | 3.0                 |
| 19         | 10.1       | 1.6                 |
| 20         | 10.5       | 6                   |
| 21         | 15.2       | 2.0                 |
| 22         | 6.4        | 3.0                 |
| 23         | 3.7        | 3.0                 |
| 24         | 12.1       | 2.0                 |
| 25         | 10.9       | 1.8                 |
| 26         | 7.8        | 2.8                 |
| 27         | 16.3       | 2.0                 |
| 28         | 9.3        | 2.0                 |
| 29         | 11.1       | 2.0                 |
| 30         | 18.7       | 0.6                 |

6 subjects is listed below and includes the operative procedures.

1 case recurrent nerve paralysis (Thyroplasty I)

1 case polyp (Polypotomy)

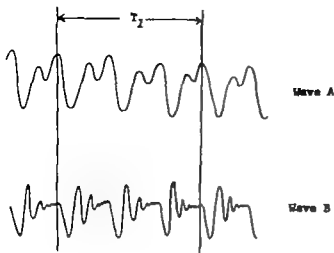
2 cases, atrophy (Thyroplasty I used in 1 case)

1 case nodules

1 case mutational voice disorder (Thyroplasty III)

In order to compare each  $R$  value with its auditory impression more clearly the score of the listener judgment was plotted against the  $R$  value for both normal and pathologic voices in Fig. 8. It was noted that there is a definite correlation between the two parameters. The calculated correlation coefficient was  $-0.868$  and significant at the 1% level. Figs 9, 10 and 11 present sonagrams for slight, moderate and severe hoarseness. The  $R_s$  values were 15.2 dB (case 21), 11.1 dB (case 29) and 4.5 dB (case 16) and these values correspond well to the visual impressions of the sonagrams.

Cases 9 and 13 show a slight deviation from the general trend. The sonagrams of these voices (Figs. 12 and 13) present sudden changes in the voice quality. Auditory impression is considered as the impression of voice over time while our method has a limitation in that it can store only 325 msec samples of

Fig 6 The calculation of  $T_1$ 

corresponding pitch periods ( $T_1$ ) of wave B (Fig 6)

#### 4 Fourier analysis

If the three pitch periods ( $T_1$ ) of wave B are repeated endlessly the result can be considered a periodic wave  $f(t)$  whose pitch is  $T_1$  where frequency is  $F=1/T_1$ . Therefore this wave  $f(t)$  can be expanded into a series of sinusoidal waves whose pitch periods are  $T_1$ ,  $T_1/2$ ,  $T_1/3$ , ...,  $T_1/n$  using Fourier Expansion as follows

$$f(t) = \frac{A_0}{2} + \sum \left( A_n \cos \frac{2n\pi}{T_1} t + B_n \sin \frac{2n\pi}{T_1} t \right) \\ = \frac{A_0}{2} + \sum X_n \sin \left( \frac{2n\pi}{T_1/n} t + E \right)$$

where

$$A_n = \frac{2}{T_1} \int_0^{T_1} f(t) \cos \frac{2n\pi}{T_1} t dt$$

$$B_n = \frac{2}{T_1} \int_0^{T_1} f(t) \sin \frac{2n\pi}{T_1} t dt$$

$$X_n = \sqrt{A_n^2 + B_n^2}$$

#### Calculation

Since the fundamental pitch of the actual voice wave is  $T_1/3$  (fundamental frequency =  $3F$ ) harmonic components appear on the spectrum

of  $f(t)$  at  $3F$ ,  $6F$ ,  $9F$  and between these harmonics are noise components. In this study the wave  $f(t)$  was expanded into sinusoidal waves with harmonic frequencies up to 5500 Hz and this determined the value of  $n$ . The signal/noise ratio of  $T_1$  was calculated as follows

$$R_1(\text{dB}) = 10 \log_{10} \frac{S_1}{N_1}$$

where

$$S_1 = X_3^2 + X_6^2 + X_9^2 + \dots$$

$$N_1 = (X_1^2 + X_2^2) + (X_4^2 + X_5^2) + (X_7^2 + X_8^2) + \dots$$

This procedure was repeated on the next three pitch periods ( $T_2$ ) and continued one after another ( $T_3$ ,  $T_4$ ,  $T_5$ , ...,  $T_m$ ) for 325 msec. In order to obtain the average  $S/N$  ratio  $R_a$  was calculated as

$$R_a(\text{dB}) = 10 \log_{10} \frac{S_1 + S_2 + S_3 + \dots + S_m}{N_1 + N_2 + N_3 + \dots + N_m}$$

## RESULTS

The  $R$  value for 28 normals ranged from 15.0 to 23.5 dB and Fig 7 shows the distribution. The critical region of normal was 15.4 to 23.3 dB at a 5% significance level and no statistical difference was found between males and females.

Table II presents the  $R$  values of patients' voices along with the auditory impression for each voice. Six subjects show values within

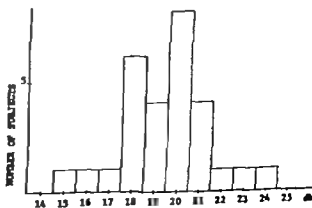


Fig 7 Histogram of normals.

This afterglow is due to the persistence of the light source.

I



Fig. 12. Sudden change in voice quality (case 9).



Fig. 13. Sudden change in voice quality (case 13).

tion or vocal shimmer was analysed by Koike (1969), von Leden & Koike (1970) and Kitzawa (1976). The previously mentioned investigators using computer analyses, have been successful in evaluating a rough quality which is related to an irregularity of the fundamental cycle.

When combined with the previous methods, the calculation of the S/N ratio of hoarse voice can distinguish a breathy voice from a rough voice. Furthermore the S/N ratio of hoarse voice obtained by the present method appears to be a quantitative indicator of the degree of hoarseness thereby providing objective information on the effectiveness of individual treatment.

## ZUSAMMENFASSUNG

Die harmonischen Bestandteile in der menschlichen Stimme werden von dem Lärme-Bestandteilen durch einen kleinen Labordigitalen-Computer abgetrennt. Das Verhältnis von Harmonie zu Lärm oder H/L-Verhältnis (S/N Ratio) wird berechnet und verglichen zu dem Gehörseindruck von 51 Personen. Die kalkulierten Resultate zeigen einen Zusammenhang zum Gehörseindruck und es wurde vorge-

schlagen, daß dieses eine hilfreiche Methode sein würde für eine quantitative Bewertung von Heiserkeit.

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Fig. 9 Sonagram of slight hoarseness ( $R_n = 15.2$  dB).



Fig 10 Sonagram of moderate hoarseness ( $R_1 = 11.1$  dB)

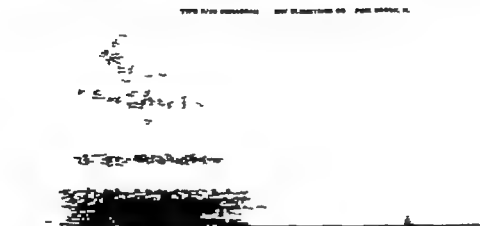


Fig. 11 Sonogram of severe hoarseness ( $R_n = 4.5$  dB).

the voice. This may be assumed to be a reason why this deviation occurred.

## DISCUSSION

There have been many attempts to establish objective parameters which represent the degree of hoarseness but the term hoarseness includes a wide range of deviation from the normal voice. Some attempts to quantify the measurement of hoarseness have dealt with auditory impression. Isshiki et al (1966, 1970) using Osgood's semantic differential method introduced a technique to classify the auditory impression of hoarse voice. As a result of his analysis he found that hoarse voice

has four factors Rough (R) Breathy (B)  
Asthenic (A) and Degree (D)

Further investigation has been done on the acoustic features of hoarseness by Hiroto (1967) using a sonograph. It was revealed that the breathy quality is characterized by a large degree of noise components and a decrease in harmonic component. The rough quality is distinguished by a great cycle to-cycle fluctuation in the fundamental frequency.

In an attempt to obtain objective parameters which represent the degree of hoarseness pitch perturbation was measured by Lieberman (1961) Iwata & von Leden (1970) Hiki (1966) and Kitajima (1975). Amplitude varia-

# LYMPHOCYTE ATPase ACTIVITY IN PATIENTS WITH CARCINOMA OF THE LARYNX

## *A Follow-up Study on 45 Patients*

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**Abstract** Mitochondria-related Mg ATPase activity was measured in lymphocytes isolated from the blood in 45 patients with epidermoid carcinomas of the larynx. Increased activity was found in 11 patients and normal or subnormal activity in the remaining cases. No correlation could be demonstrated between the magnitude or the spreading of the malignancy as recorded by the TNM classification and the ATPase activity. In 36 patients determinations were carried out repeatedly during a period from 6 to 20 months following therapy and after 3 years.

Final follow-up on all patients was done. All patients received high-voltage radiotherapy and in 7 patients additional partial or total laryngectomy was carried out, one patient had bilateral neck dissection. After radiotherapy the ATPase activity decreased significantly in 17 patients.

Half of either remained normal or increased temporarily at the first. No correlation was found between the course of the disease and the initial ATPase activity or the pattern of variations in ATPase activity following therapy. Contrary to what resulted from a preliminary study it is concluded that determination of lymphocyte ATPase activity is of no diagnostic value in patients with laryngeal carcinoma, nor does it offer any prognostic help in determining which patients may relapse or develop metastatic disease.

Increased mitochondrial lymphocyte ATPase activity has previously been found in patients with various malignancies (Ellegaard & Dimitrov 1977a, 1972b; Dimitrov & Ellegaard 1972; Dornand et al. 1976). Preliminary results from determination of lymphocyte ATPase activity in 10 patients with laryngeal carcinoma (Ellegaard et al. 1975c) have shown that half of the patients investigated had increased lymphocyte ATPase activity compared with normals and after radiotherapy this activity was normalized.

In the present study we have expanded these preliminary observations on larynx carcinoma to comprise a larger number of patients at different clinical stages of the disease. Repeated determinations of the lymphocyte ATPase activity were performed during a mean observation period of 30 months in order to establish a possible relationship between the lymphocyte ATPase activity and relapsing or metastasizing neoplasia. The results have not confirmed any relationship between the magnitude or spreading of the disease and the lymphocyte ATPase activity.

## MATERIALS AND METHODS

The study comprises a control group of 40 normal healthy subjects of both sexes (20 males, age: 18-70 years and 20 females, age: 21-86 years) and a group of 45 patients with carcinoma of the larynx: 4 females and 41 males, 34 to 86 years of age (average age 63 years). All patients had histologically proven epidermoid carcinomas of which 13 were supraglottic and 32 glottic. According to the UICC classification 6 of the patients with supraglottic tumours belonged to stage T<sub>1</sub>, 1 to stage T<sub>2</sub>, 5 to stage T<sub>3</sub> and 1 to stage T<sub>4</sub>. Of the 32 patients with glottic tumours 22 belonged to stage T<sub>1</sub>, 9 to stage T<sub>2</sub>, and 1 to stage T<sub>3</sub>. One of the patients with supraglottic tumours had regional lymph node metastases.

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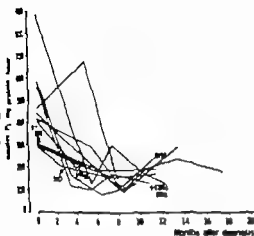


Fig. 7 Eleven patients with initially increased ATPase activity. In 10 cases the activity decreased to normal levels after radiotherapy. Four patients died within the control period and their individual survival time (in months) is indicated by figure in brackets. ND Neck dissection. M Remaining tumour mass or relapse. M Metastatic disease.

ized lymphocyte ATPase activities. Individual values are shown in Table 1 in which the staging of the tumours and the clinical response to treatment in these patients also appear. Following radiation therapy the increased ATPase activities were normalized in these patients (Fig. 1) except in one case: a patient who died before the first control. lymphocyte ATPase activity was not normalized in 2 of the patients despite per-

sisting tumour mass in one and bilateral lymph node metastases in the other.

In 7 patients (Table II) the ATPase activity rose temporarily from an initial normal level to significantly increased values (Fig. 2) after radiotherapy. This observation was not in any case related to the clinical course of the disease. Only 1 of these patients died from relapse and metastases within the control period (29 months after therapy).

The remaining 27 patients (Tables III and IV) also had normal ATPase activities before radiotherapy and the activity remained normal in 19 of these patients who were followed repeatedly after treatment (Fig. 3). In no case did the lymphocyte ATPase activities seem to be correlated to the clinical course of the disease. Three of the patients who had a remaining tumour mass after radiotherapy were operated on and they finally succumbed to their disease (Table III). Two other patients died from causes not related directly to their neoplastic disease.

After a mean observation period of 30 months 36% of the patients had relapsed among those having an initially increased ATPase activity whereas only 15% relapsed among the patients with a normal ATPase activity before therapy. However this difference is not significant ( $p > 0.2$ , Fisher's exact test).

A clinical evaluation of the patients after

Table II Seven patients with initially normal lymphocyte ATPase activity but in whom the activity later increased after therapy (Fig. 2)

a 3-year follow-up after therapy one patient (14%) had died of tumour relapse and metastases. TL Total laryngectomy PL Partial laryngectomy

| author | Age (yrs) | Sex | Tumour localization | Tumour stage                                 | Effect of treatment | Operation | Length of observation period (months) | Relapse at the end of observation | Initial ATPase activity (nmol $P_i$ /mg/h) |
|--------|-----------|-----|---------------------|--|---------------------|-----------|---------------------------------------|-----------------------------------|--|
| FAMN   | 63        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Incomplete          | TL        | 42                                    | -                                 | 153  |
| LOK    | 52        | ♂   | Supraglottis        | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | TL        | 29                                    | -                                 | 153  |
| OC     | 46        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Incomplete          | PL        | 38                                    | +                                 | 46   |
| GEB    | 77        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            |           | 43                                    | -                                 | 198  |
| VIL    | 52        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            |           | 41                                    | -                                 | 86   |
| IKB    | 86        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            |           | 44                                    | -                                 | 140  |
| AA     | 65        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            |           | 36                                    | -                                 | 263  |



Table I Eleven patients with initially increased lymphocyte ATPase activity

In 10 patients the ATPase activity permanently decreased to normal levels after therapy (Fig. 1). At a 3 year follow up 4 patients (36%) had died from tumour relapse or metastases

TL: Total laryngectomy BLND: Bilateral lymph node dissection

| Initials | Age (yrs) | Sex | Tumour localization | Tumour stage                                 | Effect of treatment | Operation | Length of observation period (months) | Relapse at the end of observation | Initial ATPase activity (nmole P <sub>i</sub> /mg <sup>1</sup> ) |
|----------|-----------|-----|---------------------|--|---------------------|-----------|---------------------------------------|-----------------------------------|--|
| RPS      | 71        | ♂   | Supraglott          | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Incomplete          | —         | 6                                     | +                                 | 383  |
| DP       | 56        | ♀   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | —         | 37                                    | —                                 | 834  |
| KAJ      | 73        | ♂   | Glottis             | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | —         | 19                                    | +                                 | 291  |
| BAVR     | 59        | ♂   | Glottis             | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | —         | 4                                     | —                                 | 419  |
| NPKK     | 82        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | —         | 38                                    | —                                 | 444  |
| JVJ      | 58        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | TL        | 25                                    | +                                 | 588  |
| HD       | 73        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | —         | 38                                    | —                                 | 301  |
| GJ       | 58        | ♂   | Supraglott          | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Incomplete          | BLND      | 42                                    | —                                 | 291  |
| EP       | 48        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | —         | 39                                    | —                                 | 467  |
| KDK      | 58        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | —         | 37                                    | —                                 | 568  |
| ASAP     | 68        | ♂   | Supraglott          | T <sub>2</sub> M <sub>0</sub> N              | Incomplete          | —         | 4                                     | +                                 | 409  |

upon presentation but none of the patients with glottic tumours had lymph node metastases. None of the patients were included in our previous study (Ellegaard et al 1975c). All the patients were treated with high-voltage irradiation (6000 rad) given within a period of 6 weeks. The result of the irradiation therapy is recorded as complete when the primary tumour was no longer visible or palpable and no signs of metastases had appeared at a clinical follow-up examination 6–12 weeks later.

Before radiation therapy blood samples were drawn from all patients for determination of the ATPase activity in circulating lymphocytes and in 37 patients blood samples were repeatedly drawn and assayed for lymphocyte ATPase activity after various intervals following the treatment. All cases were finally evaluated at a 3 year follow up and all data accumulated were analysed.

The lymphocytes were isolated from 20 ml heparinized venous blood essentially as described previously (Ellegaard & Dimitrov 1972a). Passing of the blood through a glass bead column was omitted however in order to avoid depletion of lymphocyte subpopulations e.g. of B-lymphocytes. Furthermore phagocytes were removed from the primarily

harvested suspension of mononuclear cells by the carbonyl iron method (Shah & Dickson 1974). Possibly contaminating erythrocytes were lysed by a 5 min exposure of the suspension to a 0.84% ammonium oxalate solution at 37°. The cells were suspended in cold 0.25 M sucrose (10<sup>6</sup> cells per ml) and sonicated for 2×15 sec under continuous cooling. The supernatant of the resultant homogenate was assayed for ATPase activity by the same method as previously used without modifications (Ellegaard & Dimitrov 1972a). Liberation of inorganic phosphate (P<sub>i</sub>) from Tris-ATP during incubation for one hour at 37° was determined and the results were corrected for endogenous lymphocyte P<sub>i</sub> and non enzymatic P release during the incubation. Double incubations and double determinations on each incubation were used throughout. The final results are expressed as nmole P<sub>i</sub> liberated per mg protein of the supernatant per hour.

## RESULTS

The normal controls had a mean lymphocyte ATPase activity of 210±70 (2 S.D.) nmole P<sub>i</sub>/mg/h.

Eleven patients (25%) had initially in-

Table IV Eight patients with initially normal lymphocyte ATPase activity and in whom only one ATPase determination was carried out  
 Follow-up 3 years after therapy one patient (12%) had died from tumour relapse and metastases

| Patient | Age (yr) | Sex | Tumour localization | Tumour stage                                 | Effect of treatment | Operation | Length of observation period (months) | Relapse at the end of observation | Initial ATPase activity (nmol/mg/h) |
|---------|----------|-----|---------------------|--|---------------------|-----------|---------------------------------------|-----------------------------------|-------------------------------------|
| P       | 49       | ♂   | Supraglott          | T <sub>2</sub> N <sub>2</sub> M <sub>0</sub> | Complete            | -         | 23                                    | -                                 | 170                                 |
| UP      | 84       | ♂   | Glottis             | T <sub>2</sub> N <sub>2</sub> M <sub>0</sub> | Incomplete          | -         | 10                                    | +                                 | 105                                 |
| ED      | 59       | ♀   | Glottis             | T <sub>1</sub> N <sub>2</sub> M <sub>0</sub> | Complete            | -         | 26                                    | -                                 | 165                                 |
| AK      | 54       | ♂   | Glottis             | T <sub>1</sub> N <sub>2</sub> M <sub>0</sub> | Complete            | -         | 26                                    | -                                 | 138                                 |
| LAL     | 68       | ♂   | Glottis             | T <sub>2</sub> N <sub>2</sub> M <sub>0</sub> | Complete            | -         | 26                                    | -                                 | 195                                 |
| SE      | 54       | ♂   | Glottis             | T <sub>2</sub> N <sub>2</sub> M <sub>0</sub> | Complete            | -         | 23                                    | -                                 | 104                                 |
| FC      | 65       | ♂   | Glottis             | T <sub>2</sub> N <sub>2</sub> M <sub>0</sub> | Complete            | -         | 28                                    | -                                 | 169                                 |
| SL      | 51       | ♂   | Glottis             | T <sub>2</sub> N <sub>2</sub> M <sub>0</sub> | Complete            | -         | 26                                    | -                                 | 58                                  |

Tumour cell specific sensitization of the killer cells could be due to circulating tumour cell associated antigens but non-specific killing may also be effective (Pross & Baines 1977). The energy needed for the cytotoxic potential details of which are still not understood, could be provided by hydrolysis of ATP by mitochondrial Mg ATPase. As a matter of fact it has previously been shown that a mitochondrial ATPase activity is present in human nor-

mal lymphocytes in the blood (Ellegaard & Dimitrov 1973) and that this ATPase activity is increased in patients with various neoplastic tumours including carcinoma of the larynx (Ellegaard & Dimitrov 1972a 1972b Dimitrov & Ellegaard 1972 Ellegaard et al 1975a 1975b 1975c). Contrary to what was believed from the beginning it seems to be the B-lymphocytes which carry the highest ATPase activity in unstimulated normal human lymphocytes isolated from the blood (Kragballe & Ellegaard, 1978) and elevated ATPase activity is also found in lymphocytes from patients with B-lymphocyte proliferative diseases like chronic lymphocytic leukaemia (Ellegaard, 1979).

The normal range for the lymphocyte ATPase activity is higher in the present study than in the previous investigation (Ellegaard et al 1975c). This is most likely due to alterations in the methods omitting removal of the sticky populations of cells by passing the mononuclear cell suspensions through glass-bead columns. The present study has confirmed that the ATPase activity of circulating lymphocytes is increased in a number of patients with carcinoma of the larynx and that the ATPase activity in these cases decreases to normal levels after radiotherapy. Increased protein, RNA and DNA synthesis has been demonstrated in human lymphocytes transformed

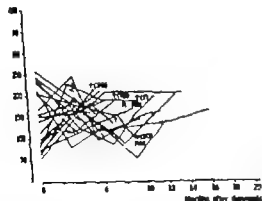


Fig. 5 Nineteen patients with initially normal or subnormal ATPase activity. After radiotherapy the ATPase activity decreased in 7 patients, while it increased in the 1 other cases. Five patients died within the control period 3 from relapse or metastases, the 2 from causes not related to their malignant disease. PL Partial laryngectomy TL Total laryngectomy R Relapse RM Relapse

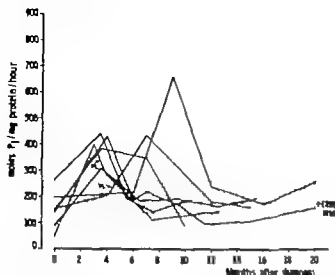


Fig 2 Seven patients with initially normal ATPase activity. After radiotherapy the activity rose temporarily but after several months the activity was again normalized in all patients. One patient died from tumour relapse and metastases (after 29 months) PL, Partial laryngectomy TL, Total laryngectomy RM, Relapse and metastases

radiotherapy did not correspond to the ATPase activity level recorded in the following control period. In the 7 patients where the treatment was recorded as incomplete the

mean of the highest ATPase activities was 264 nmol/mg/h in comparison with 259 in 29 patients where the result of the treatment was deemed complete. Similarly no difference was found between the individual mean value of all ATPase activities determined within the control period in patients belonging to either one of these groups.

It was not possible through this study to demonstrate any relationship between the survival time and either the initial ATPase activity or the average ATPase activity in the control period, as most patients were still alive at the follow up 3 years after therapy.

## DISCUSSION

Killing of neoplastic cells *in vivo* can be carried out by various cellular mechanisms which ultimately include direct action by close cell to cell contact between activated lymphocytes or macrophages and the malignant target cell (Hellström et al 1971; Periman et al 1972).

Table III Nineteen patients with initially normal ATPase activities and in whom this activity remained normal (Fig 3) during a control period varying from 7 to 20 months

At a follow-up after 3 years, 3 patients (16%) had died from tumour relapse and metastases TL, Total laryngectomy PL, Partial laryngectomy

| Initials | Age (yrs) | Sex | Tumour localization | Tumour stage                                 | Effect of treatment | Operation | Length of observation period (months) | Relapse at the end of observation | Initial ATPase activity (nmol/mg/h) |
|----------|-----------|-----|---------------------|--|---------------------|-----------|---------------------------------------|-----------------------------------|-------------------------------------|
| SPH      | 70        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 8                                     | -                                 | 260                                 |
| SQJ      | 48        | ♂   | Supraglott          | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Incomplete          | PL        | 20                                    | +                                 | 208                                 |
| TC       | 50        | ♂   | Supraglott          | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Incomplete          | TL        | 10                                    | +                                 | 208                                 |
| NREM     | 70        | ♂   | Supraglott          | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Incomplete          | TL        | 7                                     | +                                 | 83                                  |
| JKK      | 66        | ♂   | Supraglott          | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 32                                    | -                                 | 93                                  |
| HD       | 46        | ♂   | Supraglott          | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 34                                    | -                                 | 66                                  |
| LVH      | 82        | ♀   | Supraglott          | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 39                                    | -                                 | 161                                 |
| JAM      | 57        | ♂   | Supraglott          | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 31                                    | -                                 | 76                                  |
| EDP      | 34        | ♀   | Supraglott          | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 35                                    | -                                 | 244                                 |
| AMN      | 73        | ♂   | Glottis             | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 23                                    | -                                 | 177                                 |
| KAJ      | 71        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 37                                    | -                                 | 56                                  |
| KGT      | 75        | ♂   | Glottis             | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 28                                    | -                                 | 38                                  |
| HRJ      | 61        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 38                                    | -                                 | 21                                  |
| JCC      | 60        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 29                                    | -                                 | 115                                 |
| HL       | 68        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 39                                    | -                                 | 46                                  |
| SH       | 70        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 33                                    | -                                 | 147                                 |
| RFK      | 66        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 34                                    | -                                 | 155                                 |
| HJHC     | 63        | ♂   | Glottis             | T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 31                                    | -                                 | 100                                 |
| GOA      | 65        | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 30                                    | -                                 | 63                                  |

Table IV Eight patients with initially normal lymphocyte ATPase activity and in whom only one ATPase determination was carried out

Follow-up 3 years after therapy one patient (12.5%) had died from tumour relapse and metastases

| Index | Age (yr) | Sex | Tumour localization | Tumour stage                                 | Effect of treatment | Operation | Length of observation period (months) | Relapse at the end of observation | Initial ATPase activity (nanoles P/mg/h) |
|-------|----------|-----|---------------------|--|---------------------|-----------|---------------------------------------|-----------------------------------|--|
| I     | 39       | ♂   | Supraglott          | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 25                                    | -                                 | 170                                      |
| II    | 64       | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Incomplete          | -         | 10                                    | +                                 | 105                                      |
| FD    | 59       | ♀   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 26                                    | -                                 | 165                                      |
| R     | 38       | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 26                                    | -                                 | 138                                      |
| AL    | 66       | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 26                                    | -                                 | 195                                      |
| ET    | 54       | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 25                                    | -                                 | 104                                      |
| AL    | 61       | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 28                                    | -                                 | 169                                      |
| AL    | 51       | ♂   | Glottis             | T <sub>1</sub> N <sub>0</sub> M <sub>0</sub> | Complete            | -         | 26                                    | -                                 | 58                                       |

tumour cell specific sensitization of the killer cells could be due to circulating tumour cell associated antigens, but non-specific killing may also be effective (Pross & Baines, 1977). The energy needed for the cytotoxic potential effects of which are still not understood could be provided by hydrolysis of ATP by mitochondrial Mg-ATPase. As a matter of fact it has previously been shown that a mitochondrial ATPase activity is present in human nor-

mal lymphocytes in the blood (Ellegaard & Dimitrov 1973) and that this ATPase activity is increased in patients with various neoplastic tumours including carcinoma of the larynx (Ellegaard & Dimitrov 1972a 1972b Dimitrov & Ellegaard 1973 Ellegaard et al 1975a 1975b 1975c). Contrary to what was believed from the beginning it seems to be the B-lymphocytes which carry the highest ATPase activity in unstimulated normal human lymphocytes isolated from the blood (Kragballe & Ellegaard 1978) and elevated ATPase activity is also found in lymphocytes from patients with B-lymphocyte proliferative diseases like chronic lymphocytic leukaemia (Ellegaard 1979).

The normal range for the lymphocyte ATPase activity is higher in the present study than in the previous investigation (Ellegaard et al 1975c). This is most likely due to alterations in the methods omitting removal of the sticky populations of cells by passing the mononuclear cell suspensions through glass-bead columns. The present study has confirmed that the ATPase activity of circulating lymphocytes is increased in a number of patients with carcinoma of the larynx and that the ATPase activity in these cases decreases to normal levels after radiotherapy. Increased protein RNA and DNA synthesis has been demonstrated in human lymphocytes transformed

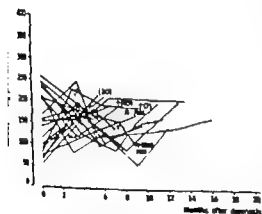


Fig. 1 Nineteen patients with initially normal or sub-normal ATPase activity. After radiotherapy the ATPase activity decreased in 7 patients, while it increased in the 11 other cases. Five patients died within the control period, 3 from relapse or metastases, the 2 from causes not related to their malignant disease. PL: Partial laryngectomy TL: Total laryngectomy R: Relapse RM: Relapse and metastases.

into a blastic stage by exposure to tumour antigens (Jehn et al 1970) or phytohaemagglutinin (Craddock et al 1971). The latter also leads to increased synthesis of phosphoproteins, decreased intracellular concentration of ATP (Kleinsmith et al 1966) and increase in the mitochondrial ATPase activity (Ellegaard & Dimitrov 1972a; Dimitrova 1977). Also we have demonstrated that exposure of normal lymphocytes to various concentrations of antilymphocyte globulin stimulates the ATPase activity proportionally (Kragballe & Ellegaard 1977). Stimulation of the lymphocyte membrane receptors by antigen-antibody complexes leading to nuclear activation, increased DNA synthesis and increased cytotoxicity against target cells was demonstrated in 1969 (Möller). Similar biochemical events could result from stimulation of circulating lymphocytes by tumour associated antigens.

However, a direct link between activation in neoplastic disease of the lymphocyte membrane receptors of which Fc-receptors seemed to be essential for the killing effect (Pross & Baines 1977; Galili & Schlesinger 1978; Kristensen & Langvad 1978; Lotzova & McCredie 1978) and the mitochondrial ATPase activity has not yet been demonstrated. The synthesis and maintenance of the lymphocyte membrane receptors is an energy requiring process (Berke & Gabison 1975; Galili & Schlesinger 1975; Buschkin et al 1975) which is also dependent on the cyclic AMP system (Chisan & Edgington 1974; Grieco et al 1976). In a previous study we found significantly higher ATPase activity in a B-cell enriched lymphocyte suspension having more Fc receptors (Kragballe & Ellegaard 1978) but in the present investigation we did not intend to measure the ATPase of different lymphocyte subpopulations.

The present study has demonstrated that determination of the lymphocyte ATPase activity in patients with laryngeal carcinoma does not add another diagnostic tool to the existing ones as significantly elevated ATPase activities were only found in 25% of all the patients

in the untreated stage and as no correlation could be found between the tumour stage and the ATPase activity. Also a decrease in the ATPase activity to normal levels was recorded in two patients despite a persisting considerable tumour mass and a rising ATPase activity was not invariably consistent with relapse or metastasizing within a control period of 30 months.

Thus the clinical value of ATPase determinations in patients with carcinoma of the larynx seems to be very slight and therefore cannot be recommended.

### ACKNOWLEDGEMENTS

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### ZUSAMMENFASSUNG

Die Aktivität der Mitochondrien-Mg-ATPase wurde in Lymphozyten, die aus dem Blut von 45 an Epidermoidcarcinom des Kehlkopfes erkrankten Patienten isoliert wurden, bestimmt. Bei 11 Patienten wurde erhöhte oder subnormale Aktivität gemessen. Große und Malignitätsausdehnung der Tumoren wurde gemäß der TNM-Klassifikation registriert; die Ergebnisse ließen sich jedoch nicht mit der ATPase-Aktivität korrelieren. Bei 36 Patienten wurden Enzymbestimmungen in einem Zeitraum von 6 bis 20 Monaten nach Therapiebeendigung durchgeführt, und nach 3 Jahren wurde eine abschließende alle Patienten umfassende Nachuntersuchung vorgenommen. Alle Patienten erhielten hoch-voltage Radiotherapie und 8 Patienten wurden zusätzlich operiert (partielle oder totale Neck-dissektion). Nach Beendigung der Strahlentherapie nahm bei 17 Patienten die ATPase-Aktivität signifikant ab, während bei den restlichen Patienten normale oder vorübergehend ansteigende Werte gemessen wurden. Es konnte keine Korrelation zwischen Krankheitsverlauf und initialer ATPase-Aktivität nachgewiesen werden; es gelang auch nicht, eine Regelmäßigkeit des Variationsmusters der ATPase-Aktivität nach Therapieabschluß aufzuzeigen. Im Gegensatz zu den Ergebnissen einer eigenen Vorstudie und wie zu dem Schluß gekommen, daß die Messung der Lymphozyten-Mg-ATPase-Aktivität weder zur Sicherung der Diagnose des Larynxcarcinoms beitragen kann, noch prognostische Aussagen hinsichtlich Rezidivhäufigkeit oder Metastasierung zuläßt.

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## AMYLOIDOSIS OF WALDEYER'S RING

*A Clinical and Ultrastructural Report*M Beiser<sup>1</sup> G Messer<sup>2</sup> J Samuel<sup>1</sup> H Gross<sup>2</sup> and E Shanon<sup>1</sup>

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(Received May 29 1979)

**Abstract** Amyloidosis of the tonsil is a rare condition and amyloidosis of Waldeyer's ring has not been previously reported. The present study describes a case of amyloidosis of the tonsil, nasopharynx and base of tongue in a 35-year-old patient in otherwise good health and without clinical symptoms. The possibility of systemic amyloidosis was excluded by clinical examination and biopsy of rectal and buccal mucosae. Electron microscopy showed the presence of typical amyloid fibrils. Large masses of the fibrils were closely surrounded by cells which appeared to be phagocytic. The ultrastructure of these cells is described. This case is considered to be a unique type of organolimited amyloidosis of Waldeyer's ring.

The term amyloid refers to a pathologic proteinaceous substance deposited in the extracellular compartment of various organs and tissues (Cohen 1967; Glenner & Page 1976). While amyloid deposits have been shown to comprise a heterogeneous group of substances, they are commonly defined by their birefringence under polarized light after Congo red staining and their characteristic fibrillar structure by electron microscopy. Studies by X-ray diffraction have indicated that such amyloid fibrils contain a  $\beta$ -pleated configuration (Glenner & Page 1976; Cohen et al. 1978; Pras & Gafni 1978).

Over the years numerous classifications of amyloidosis have been proposed based on diverse criteria. Early classification (King 1948) was based on the staining properties and divided amyloidosis into typical and atypical forms. Other classifications were based on the pathologic mechanism (Symmers 1956), histologic distribution (Heller et al. 1964) or the predominant clinical patterns (Isobe & Osser-

man 1974). The latest classification discussed by Glenner & Page (1976) represents a slight modification of that suggested by Symmer and further de-emphasizes the anatomic patterns of amyloid deposition. They make a distinction between amyloidosis which is restricted to the conditions in which major recognizable anatomic or physiologic alteration is produced by the presence of amyloid material and localized deposits of amyloid where it is present without the production of an easily recognizable clinical abnormality.

Organolimited amyloidosis was shown to be confined to various systems such as the eye (Smith & Zimmerman 1966), the urinary tract (Tirpathi & De Sauteles 1969) and the respiratory tract (Weiss 1960). Serious morbidity and even mortality may result from involvement of critical areas. The first case report on amyloidosis of the larynx dates back to 1875 (Ziegler). The symptoms and signs of amyloidosis of the respiratory tract depend on the anatomic location and may include hoarseness, hemoptysis, dyspnea, cough and stridor. The pulmonary radiological picture varies from a coin lesion, single or multiple, to diffuse stippling.

The most common oral manifestation is macroglossia. The reported incidence of this sign is 21-44% among patients presenting oral manifestations of the disease (Kenth 1972). The enlargement of the tongue is symmetrical and diffuse and in severe cases it may interfere with oral closure and cause dysphagia, dysarthria and dyspnea. Other oral manifestations

thiae and ecchymoses which tend to bleed easily. Localized deposits of amyloid are common in oral plaques, diffuse thickenings or translucent papillae. Amyloid infiltration has been reported in certain odontogenic tumors (Vickers et al 1965). The deposition of amyloid frequently precedes the calcification in the tumor.

There have been only a few reports of amyloid deposits in the tonsils (Mutschler 1933, Chan 1937, Eriksen 1970). In these, the presenting symptoms varied from no apparent disturbance to severe obstruction.

The present study describes a unique case of amyloidosis of Waldeyer's ring and electron microscopic observations of large amyloid deposits and the cells surrounding them in palatine tonsils.

## CASE REPORT

A 35-year-old male patient, born in Morocco, was admitted to our department in February 1977. Six years prior to the present admission he was treated for acute maxillary sinusitis. Three months before admission he had an attack of acute tonsillitis, which was treated successfully by ampicillin. Five weeks later another episode of tonsillar infection occurred. He was referred to our outpatient clinic because of a mild feeling of discomfort in the throat which had persisted since the last episode of infection.

On examination both tonsils were found to be enlarged and firm. Several yellowish plaques were observed on their anterior surface and an additional large plaque measuring 3.0 × 4.0 cm was seen over the left posterior pillar. Several yellowish masses occupied the base of the tongue and encroached on the epiglottis. Another mass was seen in the left vallecula. A plaque 3.0 × 0.3 cm in size spread upward from the upper border of the oropharynx to the lateral wall of the nasopharynx in the left side and another small plaque was seen in the middle of the posterior wall of the

nasopharynx. The patient was in good physical condition and examination revealed no other pathological findings.

Laboratory findings: peripheral blood hemoglobin 13.8–15.0 G% ESR (Westergren) 10 mm in the first hour; leukocytes 5700–7700 per mm<sup>3</sup> with a normal differential count. Urine analysis including microscopic examination of sediment disclosed no abnormality. Blood levels of glucose, urea, uric acid, albumin, globulin, creatinine, bilirubin, cholesterol, electrolytes, alkaline phosphatase, SGOT, transaminase, prothrombin, calcium and phosphorus were all within the normal range. Creatinine clearance was 125 ml/min, serum folate 8.8 ng/ml, serum B<sub>12</sub> 266 pg/ml. Serologic tests: Wassermann, Kahn, VDRL, cold agglutinins, Rose-Waaler, Paul-Bunnell latex, rheumatoid factor, antinuclear factor, monostest were all negative. Anti-DNA level was 12.1%. LE cells were not found. Measurement of blood immunoglobulins showed IgA 168 mg%, IgM 67 mg% and IgG 1830 mg%.

In vitro stimulation of peripheral blood lymphocytes by lectin mitogens (phytohemagglutinin, Concanavalin-A, pokeweed mitogen) was normal.

Tonsillectomy and biopsy of the nasopharyngeal and hypopharyngeal tissue masses were done. All the studies revealed amyloidosis as determined by Congo Red staining. Biopsies of rectal and buccal mucosae did not show any signs of amyloid deposits.

## METHODS

Different regions from the excised right tonsil were fixed in 2.5% glutaraldehyde in cacodylate buffer, pH 7.4, for 2 hours and post-fixed in 1% osmium tetroxide for one hour. Following dehydration in a graded ethanol series and propylene oxide they were embedded in Epon 81 (Luft 1961). Semithin sections were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate.



## AMYLOIDOSIS OF WALDEYER'S RING *A Clinical and Ultrastructural Report*

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## OBSERVATIONS

By light microscopy tonsillar tissue was seen to contain numerous amyloid deposits of varying size and shape. They appeared to impinge upon the epithelium in some instances as well as fill extensive areas of subepithelial regions. Many of the large subepithelial deposits were round to oval (in shape) and were encircled by cells many of which contained numerous vacuoles (Fig. 1) some appeared to possess several nuclei.

Electron microscopy of such regions showed the deposits comprised linear non-branching fibrils about 100 Å wide arranged in random array (Fig. 2) the characteristic ultrastructural features of amyloid fibrils (Cohen et al 1978; Glenner & Page 1976; Pras & Iftu 1978). The fibrils were closely apposed basal epithelial cells sometimes appearing to be contiguous with them. Short cytoplasmic processes extended from the cells to the fibrils (Fig. 2). Deposits also lay within deep invaginations of the epithelial cells where they were surrounded by long thin cytoplasmic recesses (Fig. 3). The basal lamina was absent in such regions and the epithelial cells were irregular in shape with only few interdigitations and desmosomes between adjacent cells.

In the subepithelial areas the large amyloid deposits were encompassed by layers of cells and cellular processes. The vacuolated cells varied in size, some reaching large dimensions (Fig. 4). They were adjoined closely to each other with a narrow intercellular gap; in some cases a clearly defined separation between apparently adjacent cells was not observable, therefore suggesting cell fusion or multiculate cells. Their cell surface was irregular with small pinocytotic-like indentations, broad undulations and numerous cell processes which extended into and around the fibrillar aggregates (Figs 4, 5, 6).

Inclusions containing fibrils similar to those in the extracellular space were observed in the cell periphery and cell processes (Figs 5, 6). They were roundish or irregular in shape

and probably represented areas of extracellular deposits which had been encompassed by the cell processes as well as grazing sections of cell invaginations enclosing extracellular fibrils. The cells also contained many heterogeneous vacuoles ranging in size from about 0.2–2 µm. They contained varying amounts of poorly defined fibrillar material, vesicles, granules, dense bodies and empty regions (Figs 6, 7) or appeared empty except for the presence of membranous and myelin-like components (Fig. 8). The cells possessed nuclei with peripheral condensed chromatin and one or more nucleoli, occasional dense lysosome-like bodies, numerous mitochondria, microfilaments, dispersed cisternae of rough endoplasmic reticulum, ribosomes and many small vesicles (Figs 7, 9).

The same amyloid deposits were surrounded also by elongated cells. Some of these cells and the long cell processes contained conspicuously large amounts of microfilaments frequently packed closely together. In some instances there was continuity between the intracellular filaments and extracellular amyloid fibrils with no intervening plasma membrane (Fig. 10). Deterioration of cells adjacent to the amyloid deposits was often observed.

## DISCUSSION

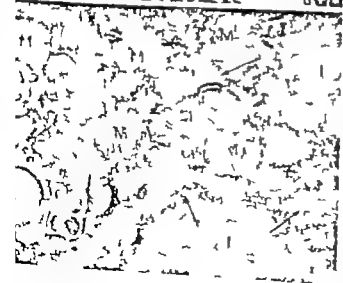
A case of amyloidosis of Waldeyer's ring is described. A thorough investigation excluded the possibility of systemic amyloidosis. The characteristic deposits were found in both palatine tonsils, base of tongue and nasopharynx. It appears that according to the criteria described by Glenner & Page (1976) this may be considered as localized organ-limited amyloidosis where the organ is the continuous lymphatic tissue comprising Waldeyer's ring. It is of interest to note that this ring of lymphatic tissue is unique in its being exposed to the external environment. Attempts were made to clarify any potential etiological factors, but no conclusions were possible.



Fig 1 Semithin epon section of large amyloid deposit (A) surrounded by cells, some of which contain numerous vacuoles.  $\times 835$

Fig 2 Electron micrograph of amyloid deposit (A) adjacent to epithelial cell (E). The amyloid fibrils are in contact with the cell periphery and cell projections (arrowheads). T tonofilaments  $\times 15225$

Fig 3 Amyloid deposit (A) in invagination of epithelial cell. Long thin epithelial cell processes (arrowhead) surround the deposit. T tonofilament  $\times 1180$



*Fig. 7* Vacuoles containing varying amounts of nuclei, granular material, small dense bodies, and empty regions. Note numerous profiles of RER (arrowhead) and small vesicles (arrow) in the cytoplasm. *M* mitochondria. 13040

*Fig. 8* Clear vacuoles, some of which contain membranous and amyloid-like components (arrow). *N* nucleus. 12180

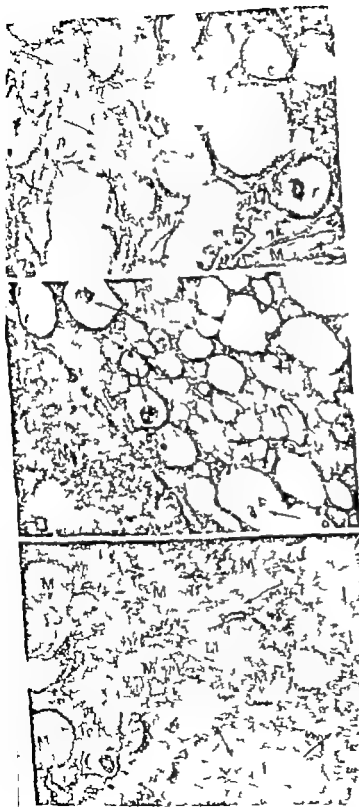
*Fig. 9* Region of acellular cell containing numerous mitochondria (*M*) and microfilaments (arrow). Amyloid inclusions. 33060



Fig. 4 Part of a large cell surrounding a big amyloid deposit (A). Cell contains nucleus (N), numerous vacuoles (V), amyloid inclusion (I) and cell processes (arrow head) extending into the deposit.  $\times 610$ .

Fig. 5 Higher magnification of amyloid deposit (A) in cell invagination and similar material in apparent cell inclusion (I). Plasma membranes appear to be absent in part of the inclusion and cell process. Clear regions between the cytoplasm and amyloid are observed.  $\times 11435$ .

Fig. 6 Vacuole containing fibrils (arrow) and sparse poorly defined fibrillar material (arrow head). A dense lysosome-like body (D) is present. A extracellular amyloid deposit.  $\times 70010$ .



*Fig 7* Vacuoles containing varying amounts of coarse granular material, small dense bodies, and empty regions. Note numerous profiles of RER (arrowheads) and small vesicles (arrow) in the cytoplasm. *M* mitochondria. 13050

*Fig 8* Clear vacuoles, some of which contain membranous and myelin-like components (arrow). *N* nucleus. 12180

*Fig 9* Region of vacuolated cell containing numerous mitochondria (*M*) and microfilaments (arrow). *I* amyloid inclusions. 33060

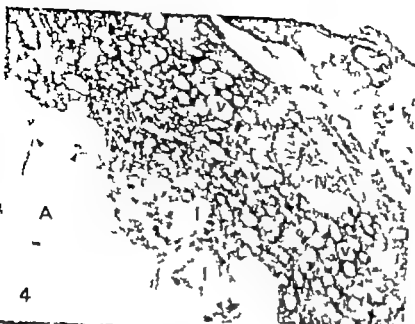


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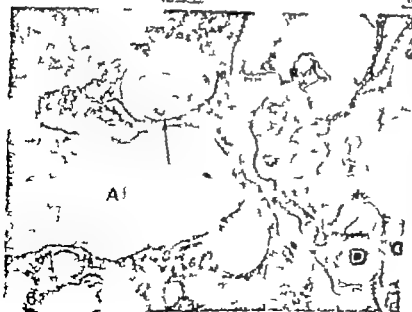


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## ZUSAMMENFASSUNG

Amyloidosen der Mundhöhle ist ein seltener Zustand und über  
 Amyloidose des Waldeyer's Rings wurde bisher nicht be-  
 richtet. In der vorliegenden Studie wird ein Fall von Amy-  
 loidosen der Mundhöhle, des Nasopharynx und der Zungen-  
 mandel bei einem 35-jährigen gesunden Patienten ohne  
 überwiegende Symptome beschrieben. Die Möglichkeit einer  
 systemischen Amyloidose wurde durch klinische Unter-  
 suchung und Biopsie von rektalen und buccalen Mucosa-  
 abschnitten. Elektronenmikroskopische zeigte das Vor-  
 handensein typischer amyloider Fibrillen. Große Mengen  
 der Fibrillen waren von Zellen, die phagocytisch erschie-  
 nen, leicht umschlossen. Die Ultrastruktur dieser Zellen  
 wird beschrieben. Bei diesem Fall handelt es sich um  
 einen eingeschränkten Typ organbeschränkter Amyloidosen  
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Fig. 10 Cell process adjacent to amyloid deposit (A). The cytoplasm contains numerous filaments (arrowheads), some in bundles (arrow). Filaments and part of cytoplasm appear continuous with the extracellular amyloid fibrils.  $\times 14,790$

Electron microscopic studies showed the presence of extensive typical extracellular amyloid deposits with close relationship to adjacent cells similar to findings described for other amyloidotic tissues (Cohen 1967; Glenner & Page 1976). Moreover cells surrounding some of the deposits may be considered phagocytes by their ultrastructural characteristics. The cellular inclusions containing fibrils likely represent endocytic vacuoles while the heterogeneous ones represent stages of digestive vacuoles active in the breakdown of the fibrils. The vacuoles observed here resemble greatly those found in experimental ultrastructural studies of phagocytic uptake and processing of amyloid fibrils by macrophages by Shirahama & Cohen (1971) and leukocytes by Zucker-Franklin (1970). Other experimental studies on amyloid resorption have indicated uptake of amyloid by multinucleated foreign body type giant cells as well as macrophages and leukocytes (Laufer et al. 1976; Polliack et al. 1970; Richter 1954; Sporn 1971; Williams 1967; Wright et al. 1972).

Ultrastructural evidence for resorption of amyloid by phagocytosis in human amyloidosis has been reported in familial amyloid nephropathy by Weiss & Page (1973). Multinu-

cleated giant cells were implicated in the phagocytic activity. They contained fibril-containing vacuoles similar in composition to those described here. Glenner & Page (1976) have noted that the mechanism of amyloid resorption in man is unknown and suggested that macrophage activity is likely to be implicated. The findings presented here support that suggestion.

Perusal of the available literature disclosed reports on three cases of amyloid deposits in the tonsils. The first case (Mutschler 1933) was a woman aged 35 with an amyloid tumor in the lower pole of the right tonsil. The second (Chian 1937) involved an almost complete amyloid degeneration of the left tonsil in a woman aged 58 years. There is only one case report on a bilateral tonsillar involvement (Eriksen 1970). Massive amyloid deposits caused an almost complete obstruction of the oropharynx. According to Glenner's classification the first case (Mutschler 1933) appears to be one of a localized deposition of amyloid. On the other hand the other two patients as well as the one observed by us represent an organ-limited amyloidosis. Our case is unique in that it involves all elements of Waldeyer's ring. This specific topography suggested an immune local response.

Table I Spontaneous cytotoxicity of tonsil and blood leucocytes of children (A) and adults (B) with chronic tonsillitis and of children with tonsillar hypertrophy (C)

| I—spontaneous cytotoxicity of tonsil leucocytes<br>II—spontaneous cytotoxicity of blood leucocytes<br>III—spontaneous cytotoxicity of blood and tonsil leucocyte mixtures |  |                      |                       |
|---|--|----------------------|-----------------------|
| Groups  | % Isotope ( $^{51}\text{Cr}$ ) release from target cells |                      |                       |
|   | I  | II                   | III                   |
| A   | $10.5 \pm 1.8$<br>=20                                    | $44.7 \pm 5.8$<br>=8 | $30.5 \pm 14.7$<br>8  |
| B   | $26.6 \pm 9.8$<br>8                                      | $35.4 \pm 11.3$<br>8 | $40.8 \pm 10.5$<br>=8 |
| C   | $12.6 \pm 1.7$<br>=20                                    | $42.3 \pm 8.7$<br>=9 | $46.6 \pm 8.7$<br>9   |

obtained by the method of Passaleva et al (1974)

Erythrocytes of chicks not more than 4 months old were used as target cells. Erythrocytes obtained in sterile conditions were treated with  $^{51}\text{Cr}$  for an hour at  $37^\circ\text{C}$ . The basic experiment was conducted as described earlier (Gjulling & Melnikov 1977)

The immunofluorescent method (Kubica, 1967) was used to elucidate the nature of cells accumulating in dog's tonsils during inflammation. Sections of experimental and control tonsils approximately  $10\ \mu$  thick were prepared on the 3rd and 8th days after inoculation. The sections were treated with immune rabbit serum obtained by means of repeated immunization of rabbits with dog thymocyte membranes. After serum depletion by bone marrow cells its lymphocytotoxic titer was 1:20 (according to 50% destruction of live fresh thymocytes)

Then sections were treated with ass serum against rabbit globulins labelled with FITC

The results were evaluated by Student's *t* test

## RESULTS

The data presented here indicate that blood cells of patients with tonsillitis destroy active

ly the chicken erythrocytes with a weak metabolic activity (Table I)

Addition of autologous tonsil cells of children with tonsillar hypertrophy to blood leucocytes increases cytolysis of heteroerythrocytes in contrast to addition of tonsils cells of patients with chronic tonsillitis which produces an inhibiting effect. Thus instead of supposed additive effect of tonsil and blood cell cytolytic activity the tonsil cells of patients with chronic tonsillitis suppress the killer function of blood leucocytes

Table II shows that spontaneous cytotoxicity of intact animal tonsil cells is comparatively not high but it increases significantly in acute and chronic tonsillitis.

Cytotoxicity of dog blood cells does not differ from that of humans: it does not change on the 3rd day and decreases slightly on the 8th day after induction of infection in tonsils

Destruction of heteroerythrocytes attains the level of an additive effect of separate tonsil and blood cell populations after addition of intact animal tonsil cells to autologous blood leucocytes. But cytotoxicity of mixed popula-

Table II Spontaneous cytotoxicity of blood and tonsil leucocytes of normal dogs (A) and animals with experimental tonsillitis on the 3rd (B) 8th (C) day after infection induction and 1 month after infectious challenge for 3 times (D)

| I—spontaneous cytotoxicity of tonsil leucocytes<br>II—spontaneous cytotoxicity of blood leucocytes<br>III—spontaneous cytotoxicity of blood and tonsil leucocyte mixtures |  |                       |                       |
|---|--|-----------------------|-----------------------|
| Groups  | % Isotope ( $^{51}\text{Cr}$ ) release from target cells |                       |                       |
|   | I  | II                    | III                   |
| A   | $8.2 \pm 4.0$<br>=5                                      | $33.0 \pm 3.0$<br>=5  | $59.6 \pm 7.29$<br>=5 |
| B   | $90.6 \pm 13.6$<br>=3                                    | $51.0 \pm 5.5$<br>=3  | $59.6 \pm 7.9$<br>=3  |
| C   | $15.6 \pm 3.0$<br>=8                                     | $40.9 \pm 10.1$<br>=3 | $26.6 \pm 10.7$<br>=3 |
| D   | $21.3 \pm 4.4$<br>=4                                     | $53.6 \pm 6.1$<br>=4  | $43.0 \pm 4.4$<br>=4  |

## TONSILLAR SUPPRESSORS OF KILLER CELLS IN TONSILLITIS

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**Abstract:** The authors studied the effect of tonsil cells obtained from intact and inflamed tonsils on spontaneous cytotoxicity of autologous blood leucocytes. It has been shown that addition of tonsil cells of patients with chronic tonsillitis and those of animals with experimental tonsillitis to blood cells with a high cytotoxic potential did not augment cytotoxicity of heteroerythrocytes but inhibited it. Meanwhile in the joint cultivation of tonsil cells of intact animals and children with tonsillar hypertrophy with autologous blood leucocytes the killer effect exceeded that seen in a separate use of these cells. The data presented in this article indicate that non-specific suppressors capable of inhibiting spontaneous killing may appear in tonsils in the course of inflammation.

Suppressor cells especially T lymphocytes were shown to be of great importance in regulation of specific and non-specific humoral and cellular immune reactions. The accumulation of these cells in particular limited the intensity of immune response in its final stage (Gershon 1975 Petrov 1976).

The increase of T cell suppressor activity in infection has been observed by Corsini et al (1977). Wolf et al (1978) revealed that human T lymphocytes which proliferated under the influence of microbial antigen produced a soluble inhibitor of cell proliferation and antibody synthesis. It seems logical to assume that if immunogenesis does not result in a complete elimination of antigen in proper time the appearance of suppressor cells may promote the transformation of an acute inflammatory process to a chronic one.

We have studied the effect of tonsil cells obtained from intact and inflamed tonsils on spontaneous cytotoxicity of autologous blood leucocytes in order to find out whether sup-

pressor activity of tonsil cells was changed in tonsillitis.

### MATERIALS AND METHODS

Tonsils and blood of 28 patients with chronic tonsillitis (8 adults aged 20 to 40 and 20 children aged 3 to 10) and those of 20 children of the same age with tonsillar hypertrophy but without clinical manifestations of tonsillitis were used.

Besides tonsil and blood cells of 5 intact 4-month-old puppies and of 10 dogs of the same age with experimental tonsillitis were used in order to study the dynamics of tonsil leucocyte suppressor activity in tonsillitis.

Tonsillar inflammation was induced by injection of 0.1 ml of mixture consisting of complete Freund's adjuvant (Calbiochem) and a 24-hour culture of group A  $\beta$ -haemolytic streptococcus. The suppressor activity was studied on the 3rd and the 8th day after infective mixture inoculation as well as 1 month after infectious challenge for 3 times at 1-1.5 month intervals.

Tonsil cell suspension was prepared as follows. Excised tonsils were washed 4 times in Hanks solution with antibiotics (penicillin and streptomycin 100 U/ml) minced with scissors and passed through a nylon filter into neutral glass bottles. The cell suspension was washed twice with Hanks solution at 4°C centrifuged (150 g 3-4 min) and diluted with 199 medium to a final concentration of  $5 \times 10^7$  cells/ml (Gjulling & Melnikov 1977). Blood leucocytes were

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tion of these cells is lower than a supposed additive effect when cells from inflamed tonsils are used

Thus the results of this study show that cells capable of suppressing the non specific killing appear in tonsils during inflammatory process development. The addition of these cells to blood cells with a high cytotoxic potential does not augment target cell cytolysis but inhibits it. Killer effect is higher in a joint cultivation of normal and hypertrophic tonsil cells with blood leucocytes than that observed in a separate cultivation of these cells.

The cells with suppressor properties are already generating in inflammation focus on the 3rd day. It seems possible that such cells may migrate from inflammation focus. An indirect evidence of this appears to be the decrease of blood cell spontaneous cytotoxicity seen in dogs on the 8th day after infectious challenge.

The immunofluorescent method shows the presence of a considerable number of fluorescent cells of lymphocyte type in tonsil sections of infected animals. It seems probable that cells which suppress spontaneous cytotoxicity are T-dependent lymphocytes.

Thus the study suggests that the frequent

recurrences of inflammation create condition for generation of suppressor cells in inflammation focus. These cells are supposed to inhibit immune response formation and to promote the chronic course of inflammation process.

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#### *Conferences and Meetings*

1980 July 9-16 Tenth International Congress on Acoustics to be held in Sydney Australia. Information: 10th ICA Congress Secretariat, GPO Box 2609 Sydney NSW Australia 2001

1980, July 20-23 International Symposium on "Neuronal Mechanisms of Hearing" to be held in Prague, Czechoslovakia. Information: J. Syka, Institute of Experimental Medicine, Czechoslovak Academy of Sciences, 128 08 Prague 2, U nemocnice 2, Czechoslovakia.

1980 September 2-5 IV International Symposium on Facial Nerve Surgery to be held in Los Angeles, California, USA. Further information address: Facial Nerve Symposium, c/o Ear Research Institute, 256 South Lake Street, Los Angeles, California 90057 USA.

1980, September 21-22 The XXXI Congress of the Polish Otolaryngological Society will be held in Poznań. Further information: As Professor Antoni Pruszyński, Clinic of Otolaryngology, Academy of Medicine, 43 Przybyszewskiego St., 50 355 Poznań, Poland.

1980, September 27-28 The Research Forum, under the joint sponsorship of the Committee for Research in Otolaryngology of the American Academy of Otolaryngology and the Association for Research in Otolaryngology will be held in Anaheim, California. Abstract format instructions from Professor Makoto Igarashi, M.D., Department of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, Texas 77030, USA.

1980 October The Ear Research Institute announces a two-week Temporal Bone Surgical Dissection Course. Information: Antonio De La Cruz, M.D., Director Temporal Bone Surgical Dissection Course, Ear Research Institute, 256 South Lake Street, Los Angeles, CA 90057 USA.

1980 October 3 A Meeting of the O.R.S. (Oto-Rhino-Laryngological Research Society) will be held at the Royal National Throat, Nose and Ear Hospital, Gray's Inn Road, London. Information: Professor P. Stell, Ch.M., F.R.C.S., Department of Otolaryngology, Royal Liverpool Hospital, Prescott Street, Liverpool. L7 8XP England.

1981 March 22-27 Second International Conference on Cholesteatoma and Mastoid Surgery to be held in Tel Aviv, Israel. Information: Professor J. Sadé, International Conference on Cholesteatoma and Mastoid Surg.  
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